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OBSERVATIONS ON THE USE OF PARA-AMINOHIPPURIC ACID (PAH) IN KIDNEY FUNCTION TESTS

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IN 1938, Smith and co-workers[1] introduced the method of measuring individual kidney functions by clearance tests; the technique involved the use of a constant infusion of the material whose clearance was being measured, in order to maintain a constant plasma concentration of this substance. In the determination of renal plasma flow (RPF) the materials used were Diodrast and, later, para-aminohippuric acid (PAH); inulin and, later, sodium thiosulfate were used for estimating glomerular filtration rate (GFR) and tubular excretory capacity (Tm).

In looking for simplifications of the test procedure, Foa and Foa[2] proposed the use of single injections of the test substances and measurement of the clearance while the plasma level of each substance was falling continuously. Landowne and Alving[3] modified this idea to enable determination of RPF, GFR, and Tm using only PAH, by giving a single large injection of this substance and having frequent periods of urine collection as the plasma concentration fell through the ranges where these functions could be determined—GFR and Tm being calculated from clearances at high plasma levels (25 to 80 mg. per cent) and RPF at low levels (1 to 10 mg. per cent). Because the time required for the fall from high concentrations to the range of 1 to 10 mg. per cent is unpredictable, depending on the kidney function which is in the process of being measured, we have used the two-dose method of Foa and Foa, but we have used only PAH as described by Landowne and Alving.

There recently have appeared several publications criticizing the method of determining RPF on the basis of a falling plasma concentration of Diodrast or PAH, because lower results are obtained by this method than by the method of maintaining a constant plasma level with continuous infusion of the substance used.

Newman and associates[4] pointed out that the clearances of Diodrast and PAH appear to fall continuously as the plasma concentrations fall through the range of 10 mg. per cent to 1 mg. per cent—the region where they should remain constant and maximal. Further, in their experiments it was found that exposure of the kidney to high concentration of these substances resulted in decreased clearance at subsequent low concentrations; the "self-depression" of

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the clearance seemed to be incompletely reversible. It was felt that factors such as delay time, arterial-venous differences in concentration, and changing urine flow were not responsible for the observed phenomena.

Brun and co-workers,[5] however, feel that the explanation for these findings does lie in the arteriovenous difference in concentration, which must necessarily exist with a falling plasma level, the arterial blood level being below the venous level. They suggest that the 2½ minutes usually allowed as delay time is considerably below the actual delay time; thus the average plasma concentration as calculated for a collection period would be lower than the true value.

These findings, if true, would invalidate the results of our own studies, so that we felt it necessary to examine the problem a little more closely. Our method has been as follows.

A dose of PAH is given calculated to produce a level of 20 mg. per cent which, after the stabilization period, has fallen to desired levels of 10 mg. per cent or less. After three fifteen-minute collection periods a second and larger dose is given to produce the concentrations needed for determination of GFR and Tm, and three more fifteen-minute collections are made. When the amount of PAH excreted, in milligrams per minute, for each collection is plotted against the average plasma concentration during that period, the resulting points are seen to give two straight lines, as shown by Landowne and Alving; the slopes of these lines are the measurements desired for RPF and GFR.

It is felt that examination of the line for RPF will explain the apparent difference in this function, as determined by falling and constant plasma levels. As in almost all graphs in biologic work, one finds that this line does not go through the origin when extrapolated, but must curve to do so. Others have calculated the clearance for individual collection periods mathematically, using the formula $\frac{uv}{p} = c^*$; i.e., they have taken the equivalent of the slope of the line from each point on the graph to the origin and considered each slope a measure of RPF. It is readily seen that this method would give a different clearance for each point, the highest usually being obtained for the point situated just below the onset of the "self-depression" of the clearance. But when the clearance is calculated from the slope of the line through all the points together, regardless of the origin, it is considerably higher, and of course constant for the range of concentration used.

To prove that this factor was responsible for the low values obtained on falling concentrations, several patients were tested by both techniques—single injection and continuous infusion.

Procedure.—Patients were examined in the postabsorptive state, lying in bed. A liter of water was administered by mouth, from one to one-half hour before starting. An injection of 3 ml. of 20 per cent PAH solution was given intravenously and a constant infusion was started of 14 ml. of 20 per cent PAH in 350 ml. of saline at the rate of 4 ml. per minute. Beginning fifteen minutes after the injection, three fifteen-minute urine collections were made. Three blood samples were taken during the period of collection. Following a rest interval of fifteen to twenty minutes, an injection of PAH was given in a dose calculated to give a concentration of 20 mg. per cent in a volume of fluid having a weight equal to

*Where u = concentration in the urine, v = volume of urine excreted per minute, p = concentration in the plasma, and c = clearance in milliliters per minute.

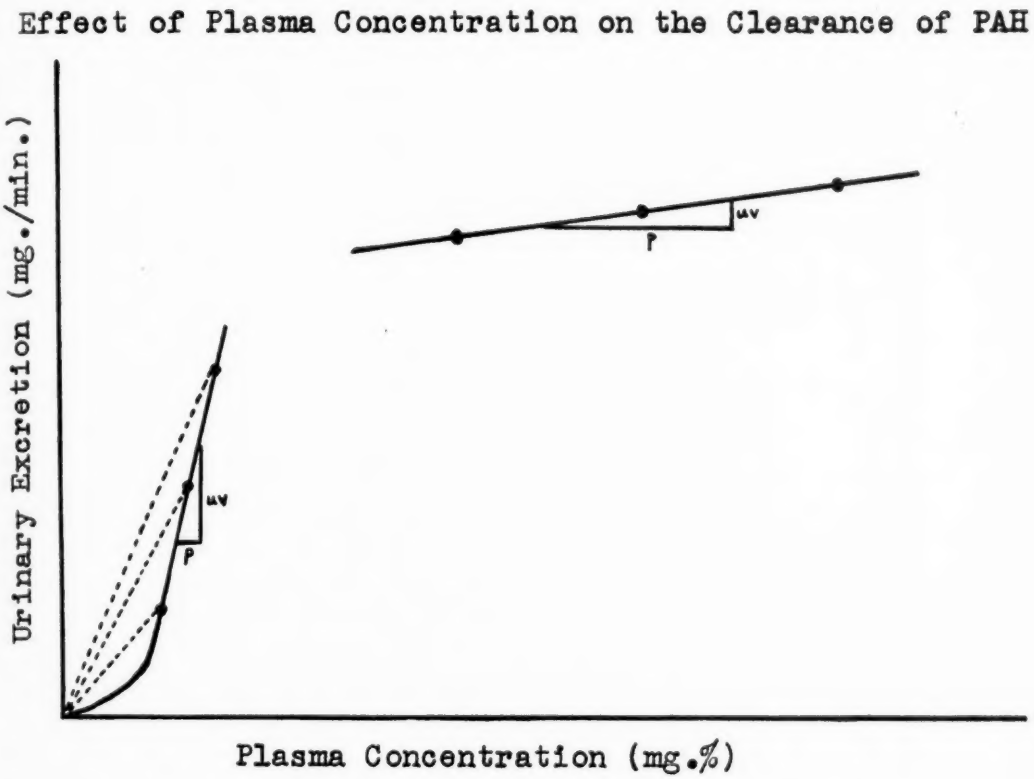

Fig. 1.—The slope of the lines ($\frac{uv}{p}$) represents PAH clearance. As the plasma concentration falls below the point of tubular saturation, there is a marked rise in clearance. The slope of the upper line represents GFR, that of the lower, RPF. The significance of the broken lines is discussed in the text.

one-fourth the body weight. This injection was given over a period of three to four minutes. Fifteen minutes after the start of the injection the bladder was emptied and rinsed. Three fifteen-minute urine collections and three more blood samples were then taken. All urine and blood samples were timed to within ten seconds.

PAH was determined by the method of Smith and co-workers.[6] The values obtained were then plotted on a graph as already discussed, and the estimations of RPF were made.

Results.—The values for RPF when estimated graphically as described are shown in Table I and Figs. 2 and 3.

TABLE I. RENAL PLASMA FLOW DETERMINED ON CONSTANT AND FALLING PLASMA CONCENTRATIONS

SUBJECT	CONSTANT CONCENTRATION				FALLING CONCENTRATION	PER CENT DIFFERENCE
	INDIVIDUAL PERIODS			AVERAGE		
J. B.	973	942	901	939	1090	+14
M. M.	1340	1295	1400	1345	1310	− 2
P. M.	603	589	592	595	582	− 3

Discussion.—Examination of the results obtained shows that there is no constant relation between the values for RPF based on constant and on falling concentrations. In two of the three subjects the results for the two methods are, for practical purposes, identical. In the remaining subject the value is

Renal Plasma Flow Determined on Constant and Falling Plasma Concentrations

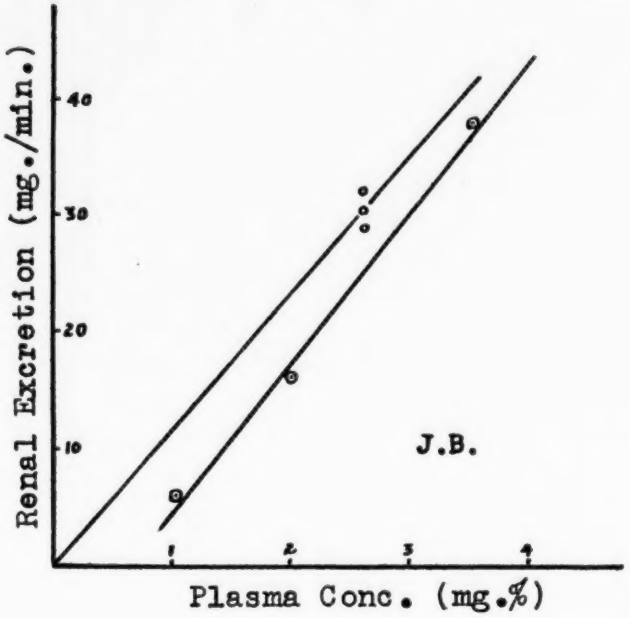

Fig.2

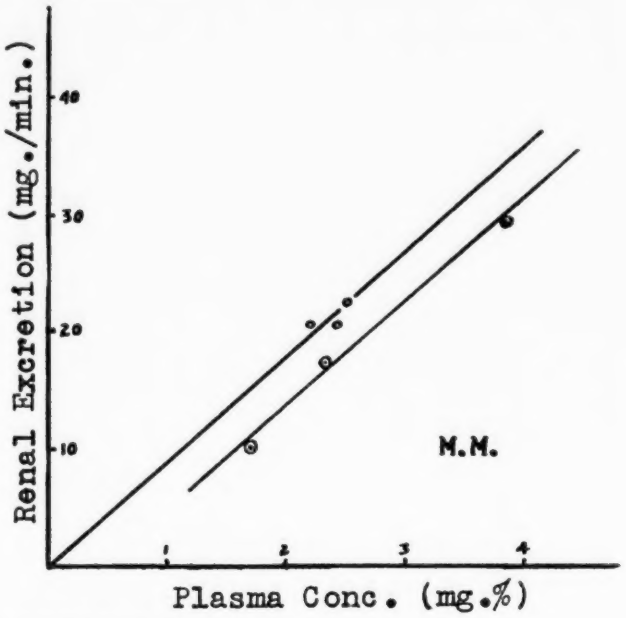

Fig.3

⊙ Falling plasma concentration
○ Constant plasma concentration

Figs. 2 and 3.—The clearance of PAH was as high when based on a falling plasma concentration as when based on a constant level; in the case of subject J. B. it was even somewhat higher.

somewhat higher for the falling concentration method, a finding quite contrary to that described by others. It may readily be seen, however, that if the clearances had been calculated mathematically for the individual collection periods by use of the formula $c = \frac{uv}{p}$, as the plasma level was falling, they would have been lower than those obtained graphically and lower than those obtained on a constant concentration, and the clearance rate would have appeared to have fallen from each urine collection period to the next.

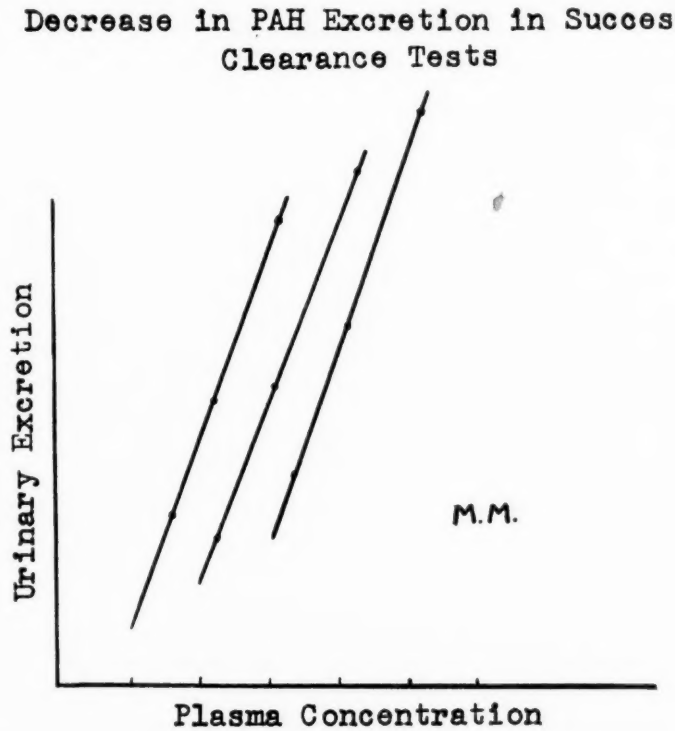

Fig. 4.—With successive determinations done at short intervals, less PAH is excreted for any given plasma concentration, though the clearance, as measured by the slope of the lines, remains unchanged.

Since the tests on falling plasma levels were made after the plasma level had initially been elevated to about 20 mg. per cent and still gave clearance rates equal to those on constant plasma levels, we may conclude that the "self-depression" is completely reversible, but that the line whose slope represents clearance is shifted to the right in the graph and does not run through the origin. It is interesting in this regard to note that when the RPF is determined several times in succession on a given subject, each line whose slope is a measure of RPF is farther from the "y" axis than the preceding one, though the slope of the lines is unchanged. (See Fig. 4.) It appears that after exposure to high concentration of PAH the tubules slacken in their efforts at excretion and

remove less of the material at a given plasma concentration than previously. The reason for this is not clear. Whether or not Tm is lowered under these conditions is a question which warrants investigation.

It was felt that these results justify the procedure of determining clearances on a falling plasma concentration with regard to reversal of self-depression. However, the problem of arteriovenous difference in concentration remained as a logical objection. To investigate this problem, it was necessary to use a method of obtaining arterial blood more easily than by repeated arterial puncture, since few patients would consent to the latter procedure. Consequently, a micromethod of PAH analysis was used allowing the use of 0.2 c.c. of finger-tip blood. The method will be described in detail elsewhere.

Procedure.—The RPF was determined in several patients, using a single injection of PAH as described, but in addition, three samples of finger-tip blood were taken during the procedure, as well as the usual venous samples.

Results.—The results of these tests are shown in Table II. It will be seen that when values for plasma concentration during the urine collection periods are uncorrected for delay time, there is little difference in the values for RPF based on venous and arterial blood. When allowance is made for delay time, there is a significant difference in results.

TABLE II. COMPARISON OF ARTERIAL AND VENOUS CLEARANCE RATES

	UNCORRECTED FOR DELAY TIME			CORRECTED FOR DELAY TIME		
SUBJECT	ARTERIAL	VENOUS	PER CENT DIFFERENCE	ARTERIAL	VENOUS	PER CENT DIFFERENCE
G. E.	615	625	0.0	580	555	+ 4.5
G. L.	845	825	+2.5	805	705	+14.3
D. N.	1130	1070	+5.6	1080	945	+14.3
M.*	615	618	0.0	600	550	+ 9.0
P. L.*	614	614	0.0			
P. J.*	540	530	+2.0			

*Data obtained from Brun and co-workers; values could not be calculated for subjects P. L. and P. J. with correction for delay time because of the lack of sufficient accurate data.

Because only a few tests could be performed in our series, the values for patients in the series of Brun and co-workers[5] have been added, after recalculation by the graphic method propounded herein, using the data in Table I and Figs. 3A, 4A, and 5A of these authors.

Discussion.—As pointed out by others, the venous plasma concentration will be higher than the simultaneous arterial plasma concentration. Thus if the plasma concentrations are plotted against time on semilog graph paper, as described by Smith,[1] two straight lines will be obtained, an upper one for venous plasma and a lower one for arterial plasma. It will be found that the arterial-venous difference in concentration is roughly constant during the greater part of the test, and this relation will be reflected in a parallelism of the lines whose slopes represent clearances if no allowance is made for delay time; the values thus obtained for arterial and venous clearance will be the same. However, if delay time is considered, a change occurs; if two and one-half minutes are allowed for venous blood, then about two minutes must be used for arterial

blood (thirty seconds for arm to kidney circulation time[7]). With this correction the lines become nonparallel and it is found that the value for venous clearance is lower than that for arterial clearance by 10 to 15 per cent. The difference between the values depends largely on the difference in concentration between arterial and venous blood; this in turn depends on RPF, which is being measured, and on the actual amount of PAH removed in each cycle through the kidney. Since both these factors are lowered in the presence of kidney disease, we may expect a decrease in the difference between arterial and venous clearances under such conditions.

It thus appears that in using clearance methods for determining kidney functions, arterial blood should be used whenever possible, since no constant correction factor can be applied to venous clearances. This is clinically practical if an arterial canula or a micromethod with capillary finger-tip blood can be used. It may be argued that the use of finger-tip blood will be inaccurate since the skin may contain more PAH than other tissues. In view of the margin of error inherent in all clearance procedures, however, this failing of the method is probably of no great significance.

SUMMARY AND CONCLUSIONS

Evidence is presented to support the validity of determining RPF on a falling plasma concentration of para-aminohippurate. At least three urine collections must be made to get a satisfactory and reliable result.

Evidence is presented to show that renal clearance as calculated on the basis of arterial blood is greater than when based on venous blood. A large series will probably show on the average a moderate increase in clearance of 10 to 20 per cent. This increase will be less with kidney damage.

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DETERMINATION OF SODIUM THIOSULFATE AND PARA-AMINOHIPPURIC ACID IN RENAL CLEARANCE TESTS

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SINCE the methods of determining specific renal functions by means of clearance techniques were introduced, there have been many modifications proposed in attempts to simplify the tests. Smith,[1] in 1934, and Richards[2] introduced the use of inulin for the measurement of glomerular filtration rate (GFR). Smith and co-workers,[3] in 1938, proposed the use of Diodrast for the estimation of renal plasma flow (RPF) and of tubular excretory capacity (Tm). Numerous changes have been made in the clearance procedures and the analytical methods in order to make the tests clinically useful. With this goal in mind, sodium thiosulfate[4, 5] and sodium P-aminohippurate (PAH)[6] were substituted for inulin and Diodrast, respectively, because of the relative ease with which they may be determined. Two improvements in analytical methods will be described, one dealing with thiosulfate, the other with PAH, which increase the utility of these valuable clinical aids.

A. THIOSULFATE

It has been possible to do simultaneous clearance tests with inulin and Diodrast, or inulin and PAH; but such has not been the case with PAH and thiosulfate, for, as shown by Elliott and Scott,[7] the presence of PAH in serum or plasma gave, by the analytical method of Newman and co-workers,[5] variable false elevations for the values of thiosulfate.

In the determination of thiosulfate by this method, addition of the reagents causes liberation of halogens; in the presence of thiosulfate less halogen is liberated. The amount of thiosulfate present is measured as the difference between the halogen liberated according to Equations I and II, the iodine being titrated with thiosulfate solution—Equation III.

$$\text{I.} \quad 2\ KIO_3 + 10KI + 12\ HCl \rightarrow 6\ I_2 + 12\ KCl + 6H_2O$$
(No thiosulfate present)

$$\text{II. (a)} \quad 2\ KIO_3 + Na_2S_2O_3 + 2\ HCl \rightarrow Na_2SO_4 + K_2SO_4 + H_2O + 2\ ICl$$
$$\text{(b)} \quad 2\ KI + 2ICl \rightarrow 2\ I_2 + 2\ KCl$$
(Thiosulfate present)

$$\text{III.} \quad 2\ Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2\ NaI$$
(Titration of halogen)

It is well known that iodine monochloride is an active halogenating agent, and no doubt the increased values obtained by this method in the presence of PAH were due to removal of some halogen by substitution, oxidation, or both. Elliott and Scott[7] found that acetyl-PAH did not consume halogen, but their attempts to acetylate the PAH prior to thiosulfate determination were unsatis-

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factory, as one might have anticipated, for it is likely that under the conditions adopted there would be only partial acetylation of the PAH and, as well, destruction of part of the thiosulfate.

Accordingly, direct titration of the thiosulfate with a standard iodine solution was investigated and it was found that interference by PAH was negligible.

Experimental.—(a) A known solution of sodium thiosulfate was made in water; varying amounts of this were added to quantities of normal plasma containing added PAH (10 mg. per cent) so that in each case the total volume was 2.0 milliliters. The concentration of thiosulfate in these solutions varied from 5 mg. per cent to 30 mg. per cent. The plasma solutions were then deproteinized by the addition of 14 ml. water, 2 ml. 5 per cent sodium tungstate, and 2 ml. 0.3N sulfuric acid. After thorough mixing and centrifugation, 15 ml. of the clear supernatant were removed to a small Erlenmeyer flask. One milliliter of 5 per cent potassium iodide and 1 drop of starch solution were added; then the solution was titrated with 0.1N iodine solution from a simple microburette of 0.2 ml. capacity made from a pipette graduated in 0.001 milliliter. A plasma blank was similarly treated. The amounts of thiosulfate were calculated from the titration values after deduction of the plasma blank titration value, allowance being made for the fact that 15 ml. portions were used for titration rather than the total original solution of 20 milliliters.

(b) To examine the stability of thiosulfate at the acidity of a protein-free blood filtrate, two solutions were made containing 1 ml. thiosulfate, 12 ml. water, and 1 ml. each of sodium tungstate solution and sulfuric acid, i.e., the amounts present in the filtrate. One was titrated at once, the other after standing one hour at room temperature.

(c) PAH to give approximately 500 mg. per cent was added to dilute urine similar to that obtained during a clearance test; 1.0 ml. of the known thiosulfate solution was added to each of several 10 ml. aliquots of this urine and after the addition of 1.0 ml. of potassium iodide solution and 2 drops of starch solution, 10 drops of 0.3N sulfuric acid were added. Each aliquot was then titrated at once with 0.1N iodine solution.

Results.—(a) In Table I the recovery of thiosulfate in plasma is compared with the amounts added. It will be seen that the values obtained by direct titration with moderately strong iodine solution are close to the true values. The range of error is −3.0 per cent to +1.3 per cent and the average error is within 0.5 per cent except for very low concentrations.

TABLE I. RECOVERY OF ADDED THIOSULFATE IN PLASMA

SODIUM THIOSULFATE ADDED (MG. %)	5.00		10.00		20.00		30.00	
SODIUM THIOSULFATE RECOVERED IN INDIVIDUAL TITRATIONS	RECOVERY (MG. %)	ERROR (%)	RECOVERY (MG. %)	ERROR (%)	RECOVERY (MG. %)	ERROR (%)	RECOVERY (MG. %)	ERROR (%)
	4.96	−0.8	9.91	−0.9	19.85	−0.75	29.60	−1.33
	4.96	−0.8	9.91	−0.9	19.85	−0.75	29.80	−0.67
	4.96	−0.8	9.91	−0.9	20.15	+0.75	30.00	+0.00
	4.86	−2.8	10.10	+1.0	20.05	+0.25	30.20	+0.67
					20.25	+1.25		
Mean	4.94	−1.2	9.96	−0.4	20.03	+0.15	29.90	−0.33

(b) The recovery of thiosulfate from the deproteinization medium is not affected by standing for as long as one hour, for the values of titration done immediately and after standing were 0.0671 ml. in each case.

(c) Satisfactory recovery of the thiosulfate was obtained in urine containing PAH, as shown in Table II. The error here is within 1 per cent.

TABLE II. RECOVERY OF ADDED THIOSULFATE IN URINE (10.00 MG. IN 10 ML.)

TITRATION	THIOSULFATE RECOVERED (MG.)	ERROR (%)
1	10.00	0.0
2	9.97	−0.3
3	10.10	+1.0
4	9.96	−0.4
5	10.05	+0.5
6	10.10	+1.0

Discussion.—It would appear that direct titration of thiosulfate with 0.100N iodine solution gives accurate and reproducible results in the presence of a very high concentration of PAH. It was not considered necessary to repeat this work in the presence of lesser amounts of PAH because even the high concentration was without interfering effect. As in all iodimetry, some practice is necessary to recognize the end point in the titration.

It is known that thiosulfate does break down in an acid medium, but apparently this process is low at the pH obtaining after deproteinization by the method used. The fact that there was no difference in titration of the thiosulfate solution after standing for one hour shows that the time factor at this stage of the procedure is negligible. If the solution must stand for longer than one hour, however, it is advisable to make it alkaline. If this is done, enough acid must be added just prior to titration to restore the pH to about 3 to 4.

Theoretically, the indirect method is eight times as sensitive as the direct. It can be seen from Equation I that 2 molecules of potassium iodate in an excess of potassium iodide and acid give rise to 12 atoms of iodine. According to Equation II, however, when thiosulfate is present as well, the same amount of iodate gives rise to only 4 atoms of iodine. In other words, 1 molecule of thiosulfate has caused the disappearance of 8 atoms of iodine. In the direct titration 1 molecule of thiosulfate used up only 1 atom of iodine (Equation III). However, such sensitivity is not essential and does not necessarily increase the accuracy. The error in the direct method is small, being much lower than that inherent in the remainder of the clearance procedure. The main advantage of the direct titration is that it makes possible the simultaneous clinical use of PAH and thiosulfate.

B. PARA-AMINOHIPPURIC ACID (PAH)

During studies on renal function, it may be necessary to do PAH determinations on arterial blood. Repeated arterial punctures are unsatisfactory; insertion of an arterial cannula, involving a "cut-down" on an artery, is rather a difficult procedure for routine use. If finger-tip blood could be used, these techniques so objectionable to the patient could be avoided. Blood obtained by finger-tip puncture is essentially capillary blood when the finger is warm and in the case of PAH is equivalent to arterial blood. PAH determination by the method of Smith and associates[6] requires 2 ml. of plasma. However, one can obtain readily only 0.2 ml. of whole blood from the finger tip because collection of more than this amount requires too much time for use in a test in which blood sampling should theoretically be instantaneous. For the use of finger-tip blood, a micro-

method for the determination of PAH had to be devised since as far as is known such a method has not yet been described. By using the protein precipitation procedure described by King,[8] which permits the use of 0.20 ml. of blood, and a slight modification of the diazotization and coupling reactions described by Smith and co-workers[6] an analytical procedure which allows the use of finger-tip blood in clearance tests has been made available.

Experimental.—

Method of Isotonic Protein Precipitation:

The reagents used are:

(a) Sodium sulfate solution, 3 per cent (crystals $Na_2SO_4 \cdot 10\,H_2O$).

(b) Zinc sulfate solution, 10 per cent (crystals $Zn\,SO_4 \cdot 7H_2O$).

(c) Sodium hydroxide solution, 0.5N.

To 0.20 ml. of whole blood are added 3.2 ml. of (a), 0.3 ml. of (b), and, last, 0.3 ml. of (c). After gentle but thorough mixing, centrifugation will separate a bright red precipitate and a clear supernatant.

To make certain that isotonic deproteinization as described does not per se interfere with the recovery of PAH, 2.0 ml. portions of blood containing PAH were treated by this method using amounts of the reagents in proportion to the volume of blood. Two milliliters of plasma were treated by the cadmium sulfate method as used by Smith and co-workers.[6] The amount of PAH was determined in each by diazotization of 10.0 ml. of protein-free centrifugate; correction was made for volume differences of the reagents and allowance was made for the hematocrit value. Recovery of PAH was identical by the two methods of deproteinization. See Table III.

TABLE III. RECOVERY OF PAH

BLOOD	CONCENTRATION OF PAH IN PLASMA (MG. %)	
	SMITH	KING
1	5.00	5.01
2	3.85	3.82
3	2.97	3.02

The complete micromethod was then tried. It consists of deproteinization of 0.20 ml. of whole blood as described and diazotization of 2.00 ml. of the protein-free supernatant. The latter step is performed in the manner of Smith and associates[6] but with one fifth the amount of each reagent. The reagents necessary for color development are:

(a) 1.2N HCl

(b) 100 mg. per cent $Na\,NO_2$.

(c) 500 mg. per cent ammonium sulfamate.

(d) 100 mg. per cent N-(1-naphthyl) ethylenediamine dihydrochloride.

To 2.0 ml. of plasma filtrate add 0.4 ml. of (a) and 0.2 ml. of (b), and mix the solution. Not before three minutes and not after five minutes, add 0.2 ml. of (c) and mix; between three and five minutes later, add 0.2 ml. of (d) and mix. A blank made of 2.0 ml. of water treated in the same manner is used to set the colorimeter at 100. Solutions are read at any time after ten minutes, using a 540 μ filter.

A standard curve was made for the spectrophotometer, using micro-cuvettes, based on 2.00 ml. aliquots of aqueous PAH solution each containing 0.0005 to 0.0070 mg. of PAH. The curve was checked several times. A 200.0 mg. per cent solution was then made of PAH in water; 0.25 ml. of this was added to 9.75 ml. of blood so that the blood contained PAH in a concentration of 5 mg. per cent. Varying amounts of this blood were added to portions of whole blood containing no PAH. The amount of PAH was determined in 0.20 ml. by the method already given, using the new standard curve. Allowance was made for the hematocrit.

Results.—

Calculations were made as in the following example:

Given: Hematocrit, 45 per cent cells.

Amount of PAH in 2.00 ml. protein-free filtrate, 0.005 mg.

The amount of plasma in the blood is 55 per cent of 0.20 ml. = 0.11 ml.

The PAH originally was dissolved in this volume.

Volume of protein precipitating reagents, 3.8 ml.

Therefore total fluid containing PAH = 3.8 + 0.11 = 3.91 ml.

2.00 ml. of this used for diazo reaction contained 0.005 mg.

Therefore total PAH = $3.91/2.00 \times 0.005$ mg.

This was originally dissolved in 0.11 c.c. plasma.

Therefore concentration = $\frac{100}{0.11} \times \frac{3.91}{2.00} \times 0.005 = 8.9$ mg. per cent.

The recovery of PAH from 0.20 ml. of blood was quite satisfactory. (See Table IV.)

TABLE IV. RECOVERY OF PAH BY MICROMETHOD (0.2 ML. WHOLE BLOOD)

PAH ADDED (MG.)	PAH RECOVERED (MG.)		PER CENT ERROR	
	INDIVIDUAL TITRATIONS (RANGE)	AVERAGE	OF INDIVIDUAL TITRATIONS	OF AVERAGE
0.00025	0.00026		+4.0	+4.0
0.00075	0.00075		0.0	0.0
0.00125	0.00120	0.00124	−4.0	−0.8
	0.00128		+2.4	
0.00150	0.00148	0.00152	−1.3	−1.3
	0.00155		+3.3	
0.00250	0.00247	0.00252	−1.2	+0.8
	0.00256		+2.4	
0.00287	0.00289		+0.6	+0.6
0.00300	0.00297		−1.0	−1.0
0.00375	0.00374	0.00372	−0.3	−0.8
	0.00370		−1.3	
0.00385	0.00378		−1.8	−1.8
0.00462	0.00455		−1.5	−1.5

Discussion.—It has been shown that little if any PAH entered the red blood cells in vitro or in vivo. However, incomplete recovery of added PAH was obtained in the presence of laked red cells.[6] Thus any protein precipitation method must be done on plasma, or on whole blood without causing hemolysis. A hypertonic solution while avoiding hemolysis would draw water from the red cells, causing a dilution of the plasma and false lowering of results. The method of King, however, avoids these errors, since the reagents used are isotonic both individually and in the mixture. The results show that recovery is satisfactory by this micromethod which combines isotonic deproteinization with a slight modification of the diazotization procedure generally used. Thus since whole blood in the amount of 0.20 ml. can be used for PAH determinations, the finger-tip procedure is clinically practicable.

The standard curve is easily reproducible. It must be in terms of milligrams of PAH in the 2.00 ml. used for diazotization rather than in concentration in plasma, in order to avoid errors due to difference in hematocrit values.

As in all methods based on the substitution of finger-tip for arterial blood, the finger must be warmed to ensure that capillary blood is obtained. "Milking" of the finger should be avoided to minimize mixing of the blood with tissue fluid. A minor advantage of this method is that the usual step of centrifuging the blood to obtain the plasma is eliminated.

SUMMARY AND CONCLUSIONS

A method is presented for determination of thiosulfate in blood and urine by direct titration with standard iodine solution. It enables the accurate estimation of thiosulfate in the presence of high concentrations of P-aminohippurate, thus permitting the simultaneous performance of renal clearance tests with these substances.

A micromethod for the determination of P-aminohippurate is presented. It enables the use of 0.20 ml. of whole blood, which is easily obtained from a puncture wound in the finger tip. Since blood obtained this way may be considered arterial with reference to PAH, this method of determination allows the estimation of arterial blood clearance without the use of arterial puncture or cannulation.

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ADMINISTRATION OF POTASSIUM TO PATIENTS WITH PROLONGED VOMITING AND DIABETIC ACIDOSIS

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THE administration of potassium to patients by routes other than the oral was regarded until recently as a questionable procedure. This belief was based on the observation that cardiotoxic effects were produced when large amounts of this drug were given to animals,[1-4] as well as on the occasional occurrence of potassium intoxication in patients.[5-17] Recent studies relating certain pathologic states to alterations in serum potassium necessitate a re-evaluation of the role of this cation as a therapeutic agent and emphasize the need for a suitable method of administration to man. With the exception of isolated case reports[30, 31, 32] in which hypopotassemia occurred in patients during therapy for diabetic acidosis, the administration of potassium has for the most part been limited to patients with familial periodic paralysis and infants with diarrhea.[18] Darrow,[18] Govan and Darrow,[19] Butler,[20b] and Burnett and Burrows[20a] have recognized the need for a method of administering potassium by the parenteral route. The solutions of potassium chloride which they recommend vary in concentration from 15 to 35 meq. per liter.

The purpose of this report is to show that (1) concentrations of potassium higher than those previously reported can be administered with benefit by the intravenous route or by hypodermoclysis to patients deficient in this cation, (2) the amount of potassium necessary to replace the potassium deficit in patients with diabetic acidosis and prolonged vomiting often equals or exceeds 11 Gm. of potassium chloride (155 meq. of potassium), (3) although the electrocardiogram is not adequate per se to diagnose hypopotassemia, its use provides a crude estimate of the serum potassium concentration which is adequate in preventing potassium toxicity during the administration of this electrolyte.

METHOD AND MATERIALS

Forty-four patients were studied; twenty-five were undergoing treatment for diabetic acidosis, and twenty-four had intestinal obstruction associated with prolonged vomiting. The diagnosis of a deficit in potassium in these patients was made by clinical, electrocardiographic, and chemical means. Hypopotassemia was suspected in any patient with prolonged vomiting due to intestinal obstruction and the potassium deficit was found to be further increased in many of these patients who received parenteral fluids in which potassium was omitted.[23, 24] The patients with diabetic acidosis had a depletion of the total body potassium. In the diabetic patients this cation was normal or elevated in the serum on admission to the hospital,[21, 25] whereas the serum concentration of potassium was decreased in those patients with prolonged vomiting. Three of the diabetic patients showed the typical signs and symptoms associated with hypopotassemia: skeletal muscle flaccidity, lowered blood pressure, bounding

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pulse, and a loud systolic precordial murmur. The other patients, both the diabetics and those with prolonged vomiting, showed only minor degrees of these manifestations, the most prominent of which were skeletal muscle weakness and/or a decrease in the blood pressure, particularly the diastolic component.

The determination of the serum potassium was made by the flame photometer.[21] Although the chemical method of diagnosing hypopotassemia is of great value in the initial diagnosis of a deficit in this cation, it was not always practical, because of time and availability, to utilize repeated determinations during the administration of potassium solutions. For these reasons serial electrocardiograms consisting of Leads I, II and III and precordial Leads CR_2, CR_3, CR_4, and CR_5 were taken. The findings of prolongation of the Q-T interval accompanied by widening of the T wave and lowering or inversion of the T waves (CR_3) were used as a crude estimate of the serum potassium concentration.[22] These criteria proved satisfactory and accurate enough for clinical use once the diagnosis of potassium depletion had been established by clinical or chemical means.

Potassium was administered by the oral, intravenous, and hypodermoclysis routes. Fifteen patients with diabetic acidosis and twenty-two with prolonged vomiting were given potassium by the intravenous route. Six patients with diabetic acidosis and two with prolonged vomiting received this drug orally. The remaining group received potassium by hypodermoclysis. When administered orally, both potassium citrate and potassium chloride were administered in amounts ranging from 2 to 18 grams. For the intravenous use, 100 to 1,000 ml. of 1.14 per cent potassium chloride were administered. For administration of this drug by hypodermoclysis, 500 ml. of a 1.14 per cent solution of potassium chloride were mixed with equal quantities of sodium chloride.

RESULTS

Optimum Time for Potassium Administration.—The optimum time to administer potassium to patients with prolonged vomiting was not difficult to decide, since the serum potassium concentration was usually decreased on admission to the hospital. Patients with diabetic acidosis, however, required special attention. The serum potassium concentration did not decrease in these patients until two to eighteen hours after insulin therapy had been instituted.[22, 23] It was at this time when the serum potassium concentrations had decreased to below normal that replacement of this cation was considered.

In most patients admitted in a state of renal insufficiency it was felt that the kidney function must be restored before potassium was administered. Exceptions to this rule were made in a small group of patients with prerenal azotemia. Table I shows typical findings in members of this group. It will be noted that

TABLE I. PATIENTS FOLLOWING PROLONGED VOMITING SHOWING ELEVATED BLOOD UREA NITROGEN DUE TO PRERENAL AZOTEMIA; ADMINISTRATION OF POTASSIUM WITH RESULTING ELEVATION OF BLOOD PRESSURE WAS FOLLOWED BY A DECREASE IN THE BLOOD UREA NITROGEN

		BLOOD UREA NITROGEN (MG. PER CENT)								
		BEFORE	AFTER 500 C.C. KCl I.V.*							
CASE	DIAGNOSIS	KCl	1	2	3	4	5	6	7	8
W. G.	Ruptured peptic ulcer	150	95			39		27		10
G. C.	Intestinal obstruction	42		21		16				
B. W.	Pyelonephritis	49	23	15	12					
J. C.	Adynamic ileus Intestinal obstruction	85	85	100	75	70 (K cit. 6 Gm.)	73	44	30	

*In these patients 500 c.c. of isotonic potassium chloride (1.14 per cent) were given intravenously daily for four days. The serum potassium was below normal prior to each period of potassium administration.

the blood urea nitrogen which was elevated before potassium administration decreased promptly after the administration of potassium in three of four patients. The improvement in kidney function followed a rise in blood pressure that accompanied potassium administration (Figs. 1, 2, and 4). This action of potassium was observed as early as 1911 by Mathison.[29] The blood pressure rise

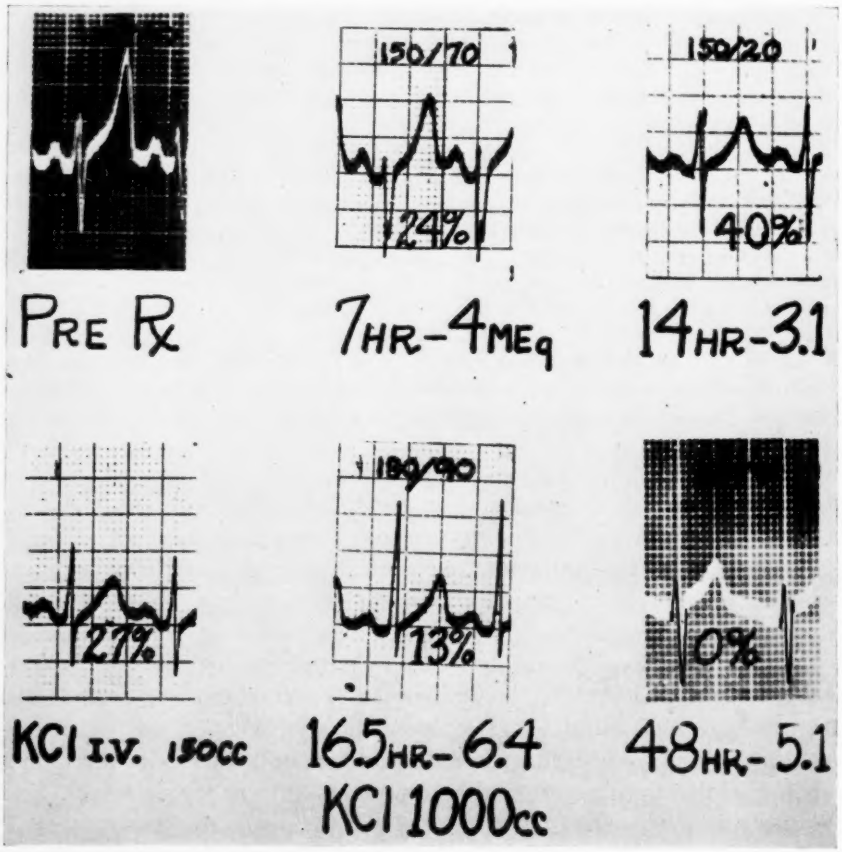

Fig. 1.—Example of the electrocardiographic changes, serum potassium concentrations, and blood pressure changes during the intravenous administration of 1,000 c.c. of potassium chloride (155 meq. per liter) during the therapy of a patient with diabetic acidosis. The potassium values are in milliequivalents per liter. The electrocardiographic changes are recorded in Lead CR_3. The per cent figures represent the percentage above normal of the Q-T interval. It will be noted that the T waves are not depressed or inverted. However, typical T-wave changes occurred in CR_4 and CR_5. The initial tracing was taken before insulin therapy and the hours are expressed as after insulin therapy.

is most striking in the diastolic component, and the administration of potassium is often accompanied by a reversal of the wide pulse pressure, bounding pulse, and systolic murmur which are often associated with the hypopotassemic state. It must be emphasized that a *low serum potassium* is a requisite if potassium is to be administered when prerenal azotemia is present.

Intravenous Administration of Potassium Chloride.—In reviewing previous reports on the use of potassium by the intravenous route, it is of interest that concentrations exceeding 36 meq. per liter of potassium have not been recommended for use in patients with hypopotassemia. Holler[30] administered a 2

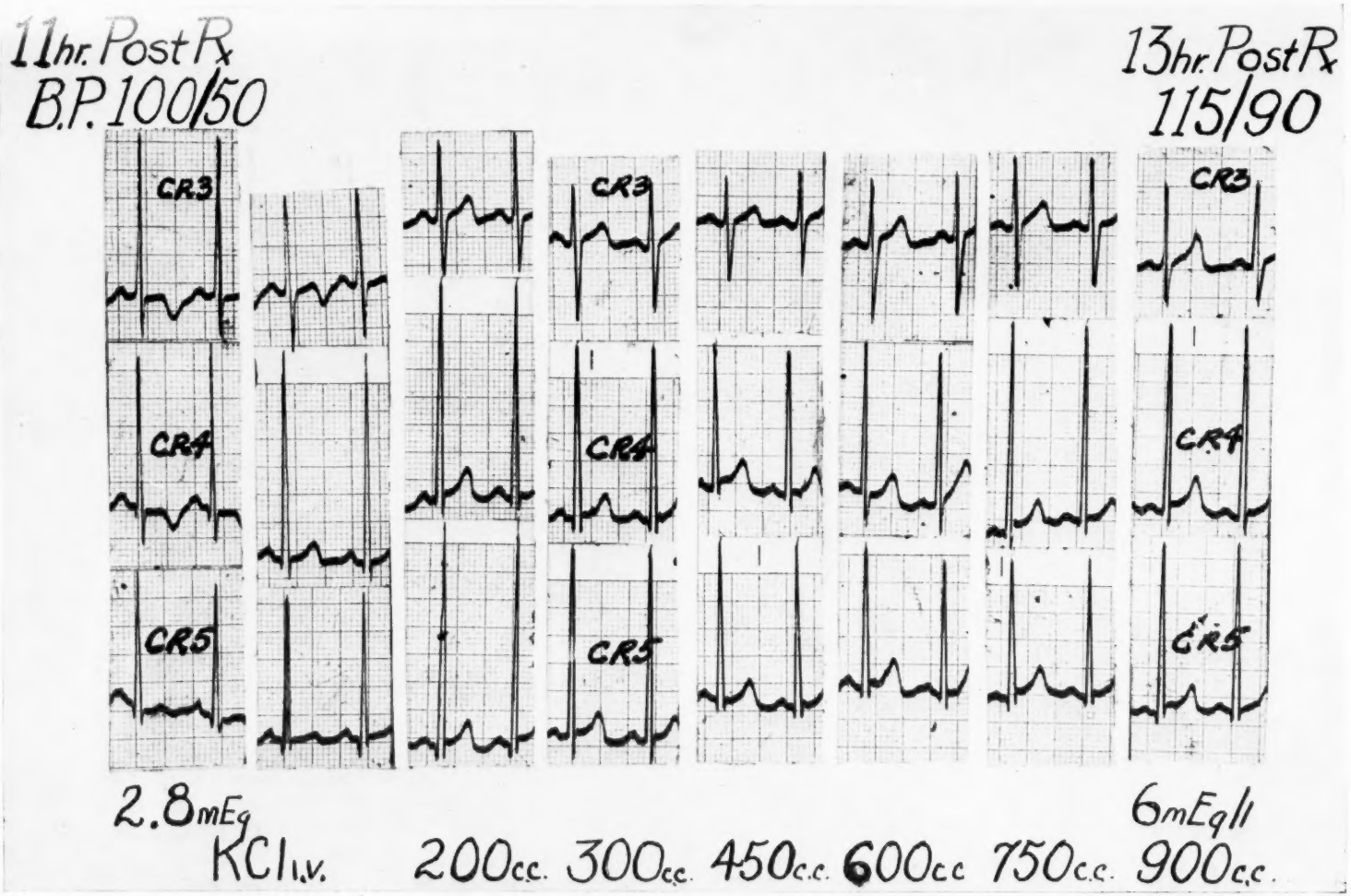

Fig. 2.—Electrocardiographic changes, serum potassium concentrations, and blood pressure changes during intravenous administration of 900 c.c. of potassium chloride during therapy of a patient with diabetic acidosis. The potassium values are expressed in milliequivalents per liter and potassium therapy was begun eleven hours after the first administration of insulin.

per cent solution of potassium chloride with success in relieving the symptoms of hypopotassemia in a single patient undergoing therapy for diabetic acidosis.[30] The 1.14 per cent solution (isotonic) used in our patients contains 155 meq. per liter of potassium. The total amount of potassium administered varied from 75 ml. to 1,000 milliliters. This represents a dosage of from 0.86 Gm. to 11.4 Gm. of potassium chloride (or 10 to 155 meq. of potassium). The maximum rate of administration to these patients was 5 ml. per minute or 3.4 Gm. of potassium chloride per hour. Generally the rate of administration varied from 3 to 5 ml. per minute. The total dosage of 11.4 Gm. was often inadequate in restoring the serum potassium to normal and supplemental potassium by other routes was required.

Although the electrocardiographic changes can be taken only as a crude estimate of the serum potassium concentration, they were found to be adequate in preventing hyperpotassemia (Figs. 1, 2, and 3). The following electrocardiographic alterations should be observed when the electrocardiogram is used as a guide to potassium effects. It will be noted that as the serum potassium concentration increases, the T waves become more upright and the Q-T interval shortens. These criteria should not be applied to patients with severe myocardial disease at this time. If after the administration of 100 ml. of potassium chloride at a rate of 3 ml. per minute the T waves do not increase in amplitude, the administration should be discontinued. On the other hand, if the Q-T interval and the T waves return to normal immediately, indicating a rapidly rising serum potassium concentration, the rate of administration of potassium should be slowed. Last, when the Q-T interval and/or the T waves assume a normal configuration, it can be assumed that the serum potassium concentration is near a normal level and the administration of potassium should be temporarily discontinued. Very often when this is done, the serum concentration of potassium will soon become lowered again, as suggested by a return of the Q-T interval prolongation and lowering or inversion of the T wave, and administration of potassium can be continued by the oral or hypodermoclysis routes. The decrease in the serum potassium which occurs when potassium administration is discontinued is apparently a consequence of both its movement into the cells[21, 34] and its excretion in the urine.[26]

Oral Administration of Potassium.—The oral administration of potassium is a safe therapeutic procedure, unless severe renal insufficiency is present. It is the treatment of choice in patients with familial periodic paralysis and other types of potassium deficiency if the patient is able to take food by mouth. Ingestion of a single dose of 12 Gm. of potassium chloride by this route in normal subjects has been shown to raise the serum concentration by 61 per cent in two hours.[34] Potassium was administered orally to the patients of this series both as a chloride and citrate salt. Usually 2 Gm. portions were administered every thirty minutes until a total of 8 to 18 Gm. had been given. In general, relatively large amounts were necessary over a period of twelve to eighteen hours before the serum potassium concentration approximated normal. Potassium by mouth was effective in reversing manifestations of potassium deficiency in the cardiac and skeletal muscle in one patient within thirty minutes after its

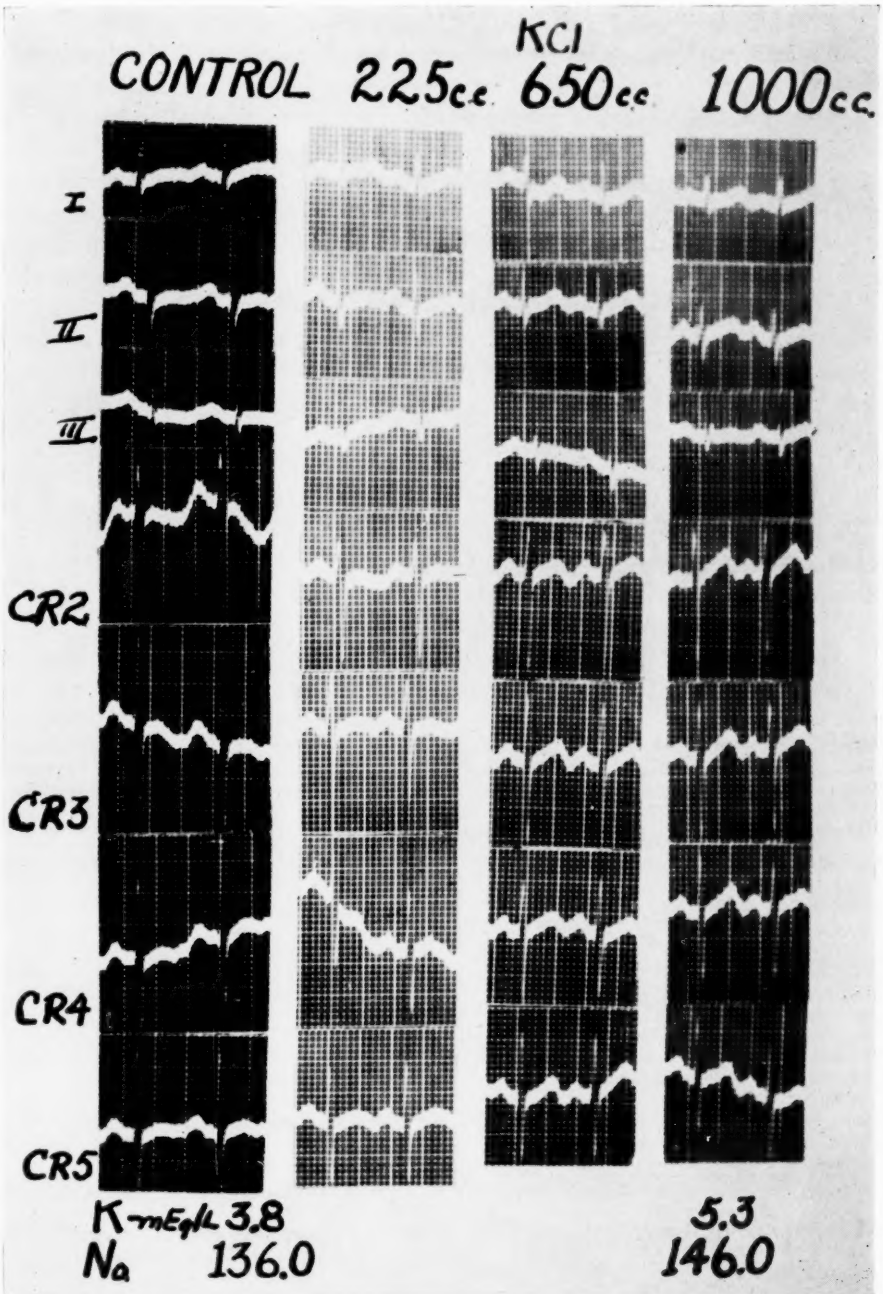

Fig. 3.—Illustrates the use of the electrocardiogram during the intravenous administration of potassium chloride in a patient following prolonged vomiting. The potassium and sodium values are expressed as milliequivalents per liter.

administration. In most patients with mild diabetic acidosis, a *soft diet* containing foods rich in potassium will correct the potassium deficit in a relatively short period of time.

Administration of Potassium by Hypodermoclysis.—Administration of potassium by hypodermoclysis was suggested by Darrow.[18, 19] It has proved to be the most satisfactory method of restoring depleted potassium and in preventing a severe decrease in the serum potassium concentration. The 1.14 per cent solution of potassium chloride can be administered as such or mixed with equal parts of physiologic saline. We have found the latter method the most satisfactory. Because the absorption of potassium chloride is slower when administered by hypodermoclysis than by vein, the estimation of the serum potassium concentration need not be as frequent as with the intravenous method. Electrocardiograms taken before and after each 500 ml. was administered proved to be adequate in safeguarding the serum potassium concentration against undue excess. Usually from 3 to 6 Gm. of potassium chloride were given initially. This was followed by a similar amount later until the patient was able to retain food.

Beneficial Effects of Potassium Administration.—The striking response to the administration of potassium of patients with diabetic acidosis and those with prolonged vomiting due to intestinal obstruction when a deficit in this cation exists requires little additional comment. A complete reversal of the skeletal muscle paralysis and cardiovascular manifestations occurs soon after potassium is given by the described methods.

Table II illustrates the change in the serum sodium after varying amounts of potassium. The rise in sodium was roughly proportional to the amount of

TABLE II. COMPARISON OF THE CHANGE IN SERUM SODIUM LEVEL AND SERUM POTASSIUM CONCENTRATIONS DURING ADMINISTRATION OF 0.6 TO 6.0 GM. OF POTASSIUM CHLORIDE IN PATIENTS WITH DIABETIC ACIDOSIS AND PROLONGED VOMITING. THE GREATEST RISE IN THE SERUM SODIUM OCCURRED DURING THE LARGER DOSES OF POTASSIUM. SODIUM WAS NOT PRESENT IN THE POTASSIUM CHLORIDE SOLUTION ADMINISTERED

DIAGNOSIS	KCl (155 MEQ./L.) ADMINISTERED INTRAVENOUSLY		CHANGE IN SERUM Na (MEQ./L.)	CHANGE IN SERUM K (MEQ./L.)
	ML.	GM. KCl		
Vomiting due to intestinal obstruction	300	2.0	+2.0	+2.0
Diabetic acidosis	300	2.0	+6.0	−1.4
Diabetic acidosis	450	2.7	+2.0	+0.3
Vomiting due to intestinal obstruction	500	3.0	+7.0	+1.7
Vomiting due to intestinal obstruction	500	3.0	+7.0	+2.8
Vomiting due to intestinal obstruction	700	4.3	+3.0	+0.8
Diabetic acidosis	*1,000*	*6.0*	*+14.0*	+0.8
Diabetic acidosis	*1,000*	*6.0*	*+6.0*	+1.4
Vomiting due to intestinal obstruction	*1,000*	*6.0*	*+10.0*	+1.5
Diabetic acidosis	*1,000*	*6.0*	*+11.0*	+3.5

potassium administered, the greatest elevations in serum sodium occurring when 1,000 ml. of potassium chloride were administered. Darrow[18] has shown that in infants with potassium depletion the sodium content of the intracellular phase is considerably increased and that administration of potassium causes a shift of sodium to the extracellular phase. A similar mechanism of sodium transfer appeared to be operating in these patients.

Toxic Effects of Potassium Administration.—Winkler, Hoff, and Smith[1] have demonstrated that toxic elevations in the serum potassium in animals cause cardiac abnormalities and eventually cardiac arrest. Occasional deaths due to

TABLE III. SERUM POTASSIUM CONCENTRATIONS BEFORE AND AFTER VARYING AMOUNTS OF POTASSIUM CHLORIDE (155 MEQ./L.) ADMINISTERED INTRAVENOUSLY TO 15 PATIENTS DURING THERAPY FOR DIABETIC ACIDOSIS. RATE OF ADMINISTRATION VARIED FROM 3 TO 5 ML. PER MIN.

SERUM K CONC. BEFORE K THERAPY (MEQ./L.)	KCl (155 MEQ./L.) ADMINISTERED INTRAVENOUSLY (ML.)	SERUM K CONC. AFTER K THERAPY (MEQ./L.)
4.4	450	4.7
4.1	500	5.2
3.2	500	6.6
3.5	500	4.0
2.6	500	4.0
2.8	900	6.0
3.8	1,000	4.6
4.1	1,000	5.5
3.1	1,000	6.4
2.5	1,000	3.2

hyperpotassemia have been reported in man.[5-17] These have been limited for the most part to patients with uremia and anuria. Tables III, IV, and V show the serum potassium concentration before and after therapy of the patients referred to in this report. The highest level of the serum potassium after the administration of this drug was 6.6 meq. per liter (Table III). We found no instance in which toxicity to the myocardium was produced in the group of patients herein reported. It must be pointed out that (one patient, Fig. 4, was an exception) the method outlined for potassium administration in our

TABLE IV. SERUM POTASSIUM CONCENTRATIONS BEFORE AND AFTER VARYING AMOUNTS OF POTASSIUM CHLORIDE (155 MEQ./L.) ADMINISTERED INTRAVENOUSLY TO 22 PATIENTS WITH HYPOPOTASSEMIA DUE TO PROLONGED VOMITING. RATE OF ADMINISTRATION VARIED FROM 3 TO 5 ML. PER MIN.

SERUM K CONC. BEFORE K THERAPY (MEQ./L.)	KCl (155 MEQ./L.) ADMINISTERED INTRAVENOUSLY (ML.)	SERUM K CONC. AFTER K THERAPY (MEQ./L.)
2.7	300	3.0
3.2	300	4.6
3.8	300	4.6
3.3	300	5.3
2.7	300	4.4
2.9	500	4.6
2.3	500	3.7
3.8	500	6.6
3.8	700	4.6
3.8	1,000	5.3

TABLE V. SERUM POTASSIUM CONCENTRATIONS BEFORE AND AFTER VARYING AMOUNTS OF POTASSIUM CHLORIDE OR CITRATE ADMINISTERED ORALLY TO 6 PATIENTS WITH DIABETIC ACIDOSIS AND 2 WITH PROLONGED VOMITING

DIAGNOSIS	SERUM K CONC. BEFORE K THERAPY (MEQ./L.)	KCl OR K CITRATE ADMINISTERED ORALLY (GM.)	SERUM K CONC. AFTER K THERAPY (MEQ./L.)	HOURS FOLLOWING K ADMINISTRATION
Vomiting	2.8	2	2.9	¾
Diabetic	4.0	6	5.0	4
Diabetic	4.0	6	2.8	12
Diabetic	3.5	8	4.1	12
Diabetic	2.8	8	5.1	12
Diabetic	3.3	12	4.3	26
Vomiting	2.3	12	2.3	12
Diabetic	3.8	16	5.9	12

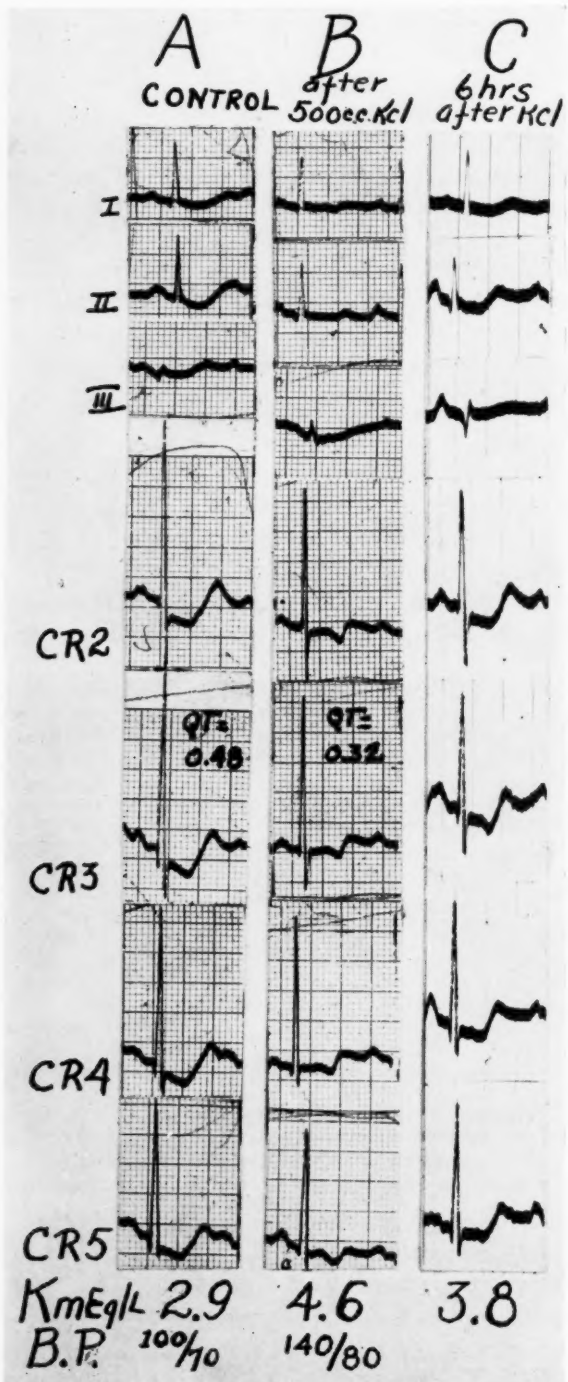

Fig. 4.—Illustrates the electrocardiographic changes, serum potassium concentrations, and blood pressure changes found before and after the intravenous administration of potassium chloride (155 meq. per liter) to a patient with prolonged vomiting and hypopotassemia after digitalization. Note that the Q-T interval shortens during potassium administration but the T wave does not become upright.

study cannot be applied to patients who have received digitalis, quinidine, or who show bundle branch block, myocardial infarction, or other evidence of severe myocardial disease. This patient, illustrated in Fig. 4, received digitalis previous to the administration of potassium. It will be noted that though the Q-T interval became shortened during potassium administration, the S-T segment remained depressed and the T wave inverted. As Tarail and Elkinton[26] have suggested, little is known about the deleterious effects of administering potassium to patients with organic heart disease. In view of the importance of this subject, experimental work was performed on the dog to determine the tolerance to potassium in various types of myocardial infarction.[33] It was found that the tolerance of dogs with small infarcts did not vary much from the normal. In those in which the infarcts were of considerable extent, a definite diminution of tolerance to potassium administration was observed.

One side effect of the administration of potassium by the intravenous route is worthy of mention. In some instances shortly after instituting this mode of therapy the patient complained of an intense burning at the site of injection. This subjective finding was often accompanied by spontaneous decrease in the rate of flow of the potassium solution. These two observations may be interpreted as indicating venospasm; however, no thrombosis or permanent vascular damage occurred in any patient treated.

DISCUSSION

The two limiting factors in the administration of potassium to patients with a deficit in this cation are (1) the state of function of the kidneys and (2) the serum potassium concentration. The absolute contraindication to the administration of potassium to a patient with poorly functioning kidneys is an established fact. Our studies suggest, however, that if the azotemia is prerenal and the serum potassium concentration is decreased, cautious administration of potassium may help relieve the shocklike state and improve renal function.

Available evidence indicates that the serum potassium concentration is the important factor both in determining the optimum time to administer potassium as well as the rate at which this cation can be administered to replace the total body deficit. It is important that potassium not be administered unless the serum concentration of this cation is decreased regardless of the total body depletions. This is based on the well-known cardiotoxic effects which accompany elevation of the serum potassium concentration. Crisman, Crisman, Calabresi, and Darrow[3] found no relationship between the intracellular potassium content of cardiac muscle and cardiotoxic effects in normal cats in which potassium chloride was administered in toxic dosages. They emphasize the correlation between the serum potassium concentration and cardiotoxic effects and emphasize that during potassium administration the important factor is that the amount given must not exceed the clearance of this cation from the blood into the intracellular space and urine. No criteria relative to the total amount of potassium needed to replace the deficit in patients with hypopotassemia has been established yet. The actual deficiency in some patients exceeds the amount of

potassium which reaches the intracellular compartment when 6 Gm. of potassium chloride are administered by the intravenous and/or hypodermoclysis routes over a period of two to three hours.

SUMMARY

Potassium was administered to forty-nine patients with hypopotassemia due to diabetic acidosis and prolonged vomiting.

The oral, intravenous, and hypodermoclysis routes were utilized. For the latter two methods a solution containing 155 meq. per liter of potassium chloride was administered. This solution was diluted with equal parts of saline in some instances for hypodermoclysis use. Orally, potassium chloride or citrate was administered.

The electrocardiographic changes associated with alterations in the serum potassium concentration were found adequate to control the rate and amount of potassium administered.

Since potassium administration is often associated with a rise in blood pressure when shocklike states are present during hypopotassemia, studies are presented which suggest that potassium administration is beneficial in prerenal azotemia if the serum potassium concentration is below normal.

A rise in the serum sodium concentration was often found when large amounts of potassium were administered.

No cardiotoxic effects were encountered even when 6 Gm. of potassium chloride were administered over a three-hour period by the intravenous route.

ADDENDUM

Since the manuscript was submitted, two patients with diabetic acidosis have been encountered who required the administration of 35 Gm. of potassium chloride in a period of twenty-four to thirty-six hours in order to replace the total body deficit of potassium. The availability of a solution containing a high concentration of potassium thus appears desirable since the total amount of fluid that would have to be administered in order to supply this much potassium chloride would be prohibitive if a solution of low potassium concentration was used.

We wish to thank Miss Mary Lanning and Miss Claire Grobman for their assistance in this study.

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EFFECT OF CITRATE SALTS AND OTHER CHEMICAL FACTORS ON THE DISTRIBUTION AND EXCRETION OF BERYLLIUM

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INTRODUCTION

EVIDENCE of the toxicity of beryllium and its compounds has been accumulating rapidly during the past few years[1-4] and has stimulated many investigations of their physiologic and biochemical behavior. Radioactive beryllium, Be^7, has been employed recently to facilitate metabolic studies in experimental animals.[5, 6, 7] The present study was directed toward clarifying the factors governing the metabolism of beryllium compounds, increasing their rate of elimination, and minimizing their deposition in the skeleton, liver, and kidneys. We have utilized the chemical complexing action of the citrate anion and the displacing or carrier action of the cations of calcium, sodium, aluminum, zirconium, and thorium.

EXPERIMENTAL PROCEDURES

General.—Young adult female Sprague-Dawley rats and an adult male dog were injected either intraperitoneally or intravenously with a beryllium salt, made with carrier-free Be^7, and at a specified time thereafter with a metal salt. The animals were kept in individual metabolism cages from which regular collections of urine and feces were made. They were sacrificed with Nembutal and several tissues were removed for beryllium analysis. The remainder of the animal (the carcass) was usually analyzed to permit complete recovery of the injected Be^7.

Preparation of Solution.—A carrier-free beryllium tracer solution was prepared in the manner described by Crowley, Hamilton, and Scott.[6] The Be^7 was manufactured by the bombardment of lithium with 10 mev protons on the 60-inch Berkeley cyclotron at the Crocker Radiation Laboratory of the University of California. The purified tracer was stored in 6M HCl. The radiochemical properties corresponded to those described previously.[6]

The solutions of metal citrates were prepared to remain stable over a wide range of pH. In each case the minimum amount of citrate necessary to prevent or minimize precipitation of the metal hydroxide was predetermined semiquantitatively. A 5 per cent solution of sodium citrate was prepared by dissolving a weighed amount of $Na_3C_6H_5O_7 \cdot 2H_2O$ in distilled water. Zirconium citrate containing 12.5 mg. as Zr. per cubic centimeter was prepared by dissolving zirconyl chloride in sodium citrate.[8] Thorium citrate was made by adding 1.9 Gm. of sodium citrate to a solution containing 1.8 Gm. of thorium chloride and diluting to give a solution containing 5 mg. as Th per cubic centimeter. Aluminum citrate was prepared by adding 50 c.c. of 12½ per cent sodium citrate solution to 18 c.c. of a solution of $AlCl_3 \cdot 6H_2O$ containing 41.6 mg. of Al per cubic centimeter. The solution was adjusted to a pH of 7 with 10 per cent NaOH and diluted to 300 c.c. to give a solution containing 2.5 mg. as Al per cubic centimeter. The calcium gluconate was a 10 per cent solution containing 9 mg. as Ca per cubic centimeter.

Methods of Analysis.—For Be^7 analysis, small tissues were placed directly in 10 c.c. porcelain capsules, covered with concentrated nitric acid, and allowed to stand at room temperature until completely dissolved. They were then dried by overhead heating with an infrared bulb. Larger tissues were wet ashed with nitric acid and then diluted with acid

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to 10 or 25 c.c., or, in the case of whole rat carcasses, to 500 cubic centimeters. Measured aliquots of these solutions were deposited in 10 c.c. porcelain capsules and dried. The radioactivity was determined by counting under an end mica-window counter tube through 500 mg. per square centimeter of a lead absorber. The activity of each tissue was compared with that of a measured sample from a dummy injection solution to which was added equivalent ashed tissue from an uninjected animal.* The dummy solution consisted of a sample of the injection fluid, equivalent in amount to that given each rat, which was deposited in a volumetric flask at the time of injection.

RESULTS

Beryllium Chloride.—

Intraperitoneal Injection: Fifteen rats weighing from 231 to 241 grams were injected with a carrier-free Be^7 solution made isotonic with NaCl and adjusted to a pH of 5. Each rat received about 300 μc of Be^7, or about 40×10^3 c.p.m. under our counting conditions. The animals were divided into five groups of three rats each. Beginning either two hours or six days later, four groups were injected intraperitoneally with either 2 c.c. of a 5 per cent sodium citrate solution or with 2 c.c. of a zirconium citrate solution containing 12.5 mg. as Zr for a total of three injections at twenty-four hour intervals. The control group received Be^7Cl_2 only. All rats were sacrificed twelve days after the Be^7 injection.

The urinary excretion of Be^7 by the control rats was about 13.6 per cent of the injected dose during the first twenty-four hours (Fig. 1). Subsequently, it

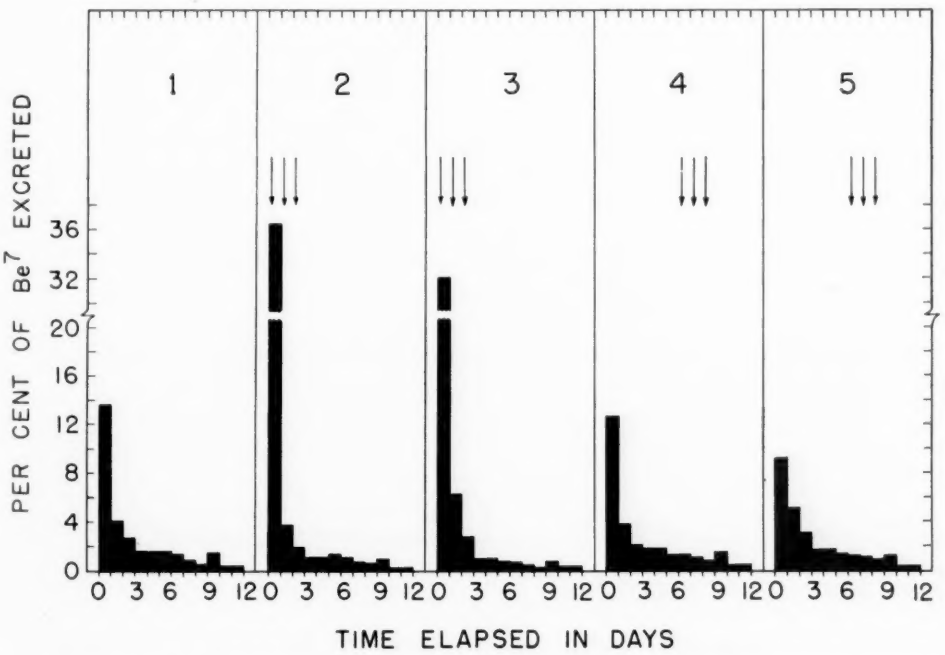

Fig. 1.—The effect of zirconium and sodium citrate administration on the urinary excretion of carrier-free Be^7 injected intraperitoneally as beryllium chloride in rats. All rats were sacrificed twelve days after injection. The numerals in the panels represent the following treatments: *1*, no treatment; *2*, zirconium citrate, beginning two hours after Be^7 injection; *3*, sodium citrate, beginning two hours after Be^7 injection; *4*, zirconium citrate, beginning six days after Be^7; *5*, sodium citrate, beginning six days after Be^7.

*Without the addition of sufficient solid matter, the measured activity of the Be^7 may be as much as 20 per cent below that of samples containing a solid residue.

decreased to about 0.5 per cent each day. Zirconium or sodium citrate administration beginning two hours after the Be^7 chloride injection increased urinary excretion to 36 and 32 per cent of the injected dose, respectively, during the first twenty-four hours but had little effect thereafter. Administration of the citrates beginning six days after the Be^7 had no significant effect upon the tissue distribution or urine content of Be. Fecal excretion was unaffected in all treated groups. At sacrifice twelve days after the injection of Be^7 about 40 per cent of the injected dose of Be had been excreted in urine and feces of control rats and the retained Be was located primarily in the bone, liver, and pancreas (Table I). In rats receiving zirconium citrate beginning two hours after the Be injection, the increased Be^7 in the urine was derived primarily from the skeleton. The increased excretion following the corresponding sodium citrate treatment, however, was accompanied by a marked reduction of the Be^7 content of the liver, spleen, and kidneys (Table I, Fig. 2).

TABLE I. EFFECT OF SODIUM AND ZIRCONIUM CITRATE ON THE DISTRIBUTION AND EXCRETION OF CARRIER-FREE Be^7 INJECTED AS Be^7Cl_2

	UNTREATED CONTROLS (%* PER ORGAN)	EARLY ZIRCONIUM CITRATE TREATED (% PER ORGAN)	EARLY SODIUM CITRATE TREATED (% PER ORGAN)	LATE ZIRCONIUM CITRATE TREATED (% PER ORGAN)	LATE SODIUM CITRATE TREATED (% PER ORGAN)
Carcass	35.7 ±1.9†	24.9 ±0.91	33.1 ±0.43	37.6 ±3.3	35.3 ±2.5
Femur	0.90 ± 0.13	0.67 ± 0.04	1.2 ±0.1	0.83 ± 0.06	0.77 ± 0.04
Liver	16.0 ±5.3	14.0 ±2.5	6.6 ±0.5	16.0 ±2.7	15.7 ±0.2
Pancreas	5.4 ±0.9	3.0 ±0.2	4.4 ±0.4	5.2 ±0.5	5.9 ±0.7
Spleen	0.82 ± 0.22	0.72 ± 0.07	0.36 ± 0.05	0.82 ± 0.07	0.96 ± 0.26
Mesenteric lymph node	0.13 ± 0.02	0.13 ± 0.02	0.14 ± 0.01	0.17 ± 0.02	0.15 ± 0.06
Kidneys	0.43 ± 0.01	0.54 ± 0.05	0.25 ± 0.04	0.61 ± 0.11	0.39 ± 0.07
Lung	0.13 ± 0.02	0.21 ± 0.07	0.10 ± 0.02	0.16 ± 0.02	0.17 ± 0.01
Total urine	30.4 ±5	50.1 ±9	46.7 ±7	29.3 ±3	27.7 ±3
Total feces	10.3 ±3	6.3 ±2	7.3 ±3	9.1 ±2	12.7 ±4

*Per cent refers to per cent of the injected dose of Be^7. The recoveries of Be^7 varied from 95.4 to 99.6 per cent. The results in the table all have been equated to 100 per cent recovery.

†Each numerical result is the average obtained from three rats and is followed by the average deviation.

Intraperitoneal versus Intravenous Injection: The relatively high concentration of Be^7 in the pancreas noted after intraperitoneal injection was not observed after intravenous or intramuscular injection.[5, 6] Accordingly, two groups of four rats each were injected with almost identical Be^7Cl_2 solutions. One group received a single intraperitoneal injection of 2.2 c.c. of an isotonic carrier-free Be^7 chloride solution adjusted to a pH of 6. The second group received a single injection via the tail vein of 0.25 c.c. of a Be^7 solution identical to the first except for the specific activity of Be^7. Each rat received about 12 μc of Be^7 or 1,500 c.p.m. under our counting conditions. The rats weighed between 221 and 234 grams. Two rats from each group were sacrificed at six and twelve days after the injection. In rats receiving intravenous injection, much more Be^7 was located in the bone, liver, and spleen than in those injected intraperitoneally, but no Be^7 was detectable in the pancreas (Table II). On the other hand, the pancreas content of the rats injected intraperitoneally was about 16 per cent of the injected dose at six days and 13 per cent at twelve days.

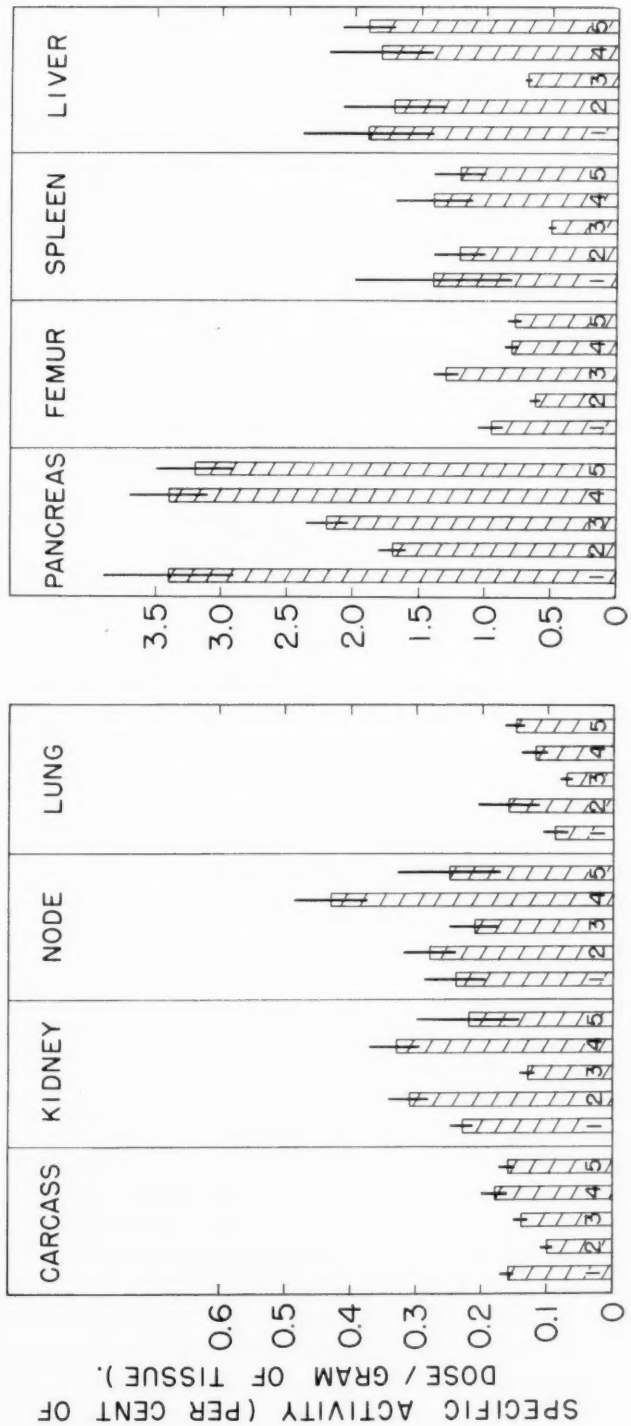

Fig. 2.—The effect of zirconium and sodium citrate administration on the tissue concentration of carrier-free Be^7 injected intraperitoneally as the chloride in rats. All rats were sacrificed twelve days after injection. The numerals inside the bars represent the following treatments: *1*, no treatment; *2*, zirconium citrate injections at two, twenty-four, and forty-eight hours after Be^7 injection; *3*, sodium citrate at two, twenty-four, and forty-eight hours after Be^7; *4*, zirconium citrate at six, seven, and eight days after Be^7; *5*, sodium citrate at six, seven, and eight days after Be^7. The vertical lines represent the average deviation of the three rats of each group.

TABLE II. THE DISTRIBUTION AND EXCRETION OF INJECTED CARRIER-FREE Be^7 INJECTED AS Be^7Cl_2 AS AFFECTED BY THE ROUTE OF ADMINISTRATION

	INTRAPERITONEAL INJECTION			INTRAVENOUS INJECTION		
TISSUE	DAY 1	DAY 6 (%* PER ORGAN)	DAY 12 (% PER ORGAN)	DAY 1	DAY 6 (% PER ORGAN)	DAY 12 (% PER ORGAN)
Femur		1.3 ± 0.1†	1.3 ± 0.1		2.4 ± 0.5	2.3 ± 0.5
Liver		5.0 ± 1.0	3.0 ± 0.6		60.0 ± 12.0	50.0 ± 10.0
Pancreas		16.0 ± 1.0	13.0 ± 2.0		<0.1	<0.1
Spleen		1.0 ± 0.1	0.9 ± 0.1		11.0 ± 3.0	6.0 ± 1.0
Mesenteric lymph node		<0.1	<0.1		<0.1	<0.1
Kidneys		<0.2	<0.2		<0.2	<0.2
Urine	19.0 ± 1.0			15.0 ± 5.0		
Feces	1.0 ± 0.4			2.0 ± 1.0		

*Per cent refers to the per cent of the injected dose of Be^7.

†Each figure represents the average from two rats, except the figures for urine and feces which are the averages from four rats. Each value is followed by the average deviation.

This concentration of Be^7 in the pancreas may be the result of the alkalinity of the pancreatic secretions. During passage of the relatively uncomplexed Be^7 from the peritoneal cavity into the blood stream following injection, it is probable that the Be^7 absorbed through the pancreas is precipitated and retained in part by the tissues. This phenomenon seems typical for hydrolyzable elements injected in an uncomplexed state.

It will be noted that the distribution of Be^7 following intraperitoneal injection differs in these two experiments (Tables I and II). In the latter a greater per cent is located in the pancreas and femur (probably in the marrow), less in the liver and kidneys. A difference in particle size distribution of the Be colloids of the two solutions used is probably responsible; Dobson and co-workers[9] have clearly shown that the size of colloidal particles greatly influences their distribution and excretion. It is difficult to prepare uniform solutions of hydrolyzable elements in the absence of complexing agents and at a pH close to the precipitation point.

Beryllium Citrate.—

Intraperitoneal Injection in Rats: Twelve rats, weighing from 170 to 180 grams, were given an intraperitoneal injection of 2.0 c.c. of a solution of 1 per cent citric acid containing Be^7 and adjusted to a pH of 7. Each rat received about 33 μc of Be^7, equivalent to 4.6×10^5 c.p.m. under our counting conditions. Two hours later, nine of the rats were given an intraperitoneal injection of a metal salt as follows: sodium citrate, 2.8 c.c. of a 5 per cent solution, two rats; calcium gluconate, 15.3 mg. per kilogram, one rat; aluminum citrate, 7.5 mg. per kilogram, two rats; zirconium citrate, 35 mg. per kilogram, two rats; and thorium citrate, 5 mg. per kilogram, two rats. Five days later all rats except those receiving aluminum were given a second injection identical to the first. All rats were sacrificed eight days after the Be^7 injection.

The urinary excretion of Be^7 in the first twenty-four hours after injection was about 40 per cent of the injected dose (Table III), as compared to only 13.6 per cent following Be^7 chloride. Subsequently it dropped to the same level (0.5 per cent each day). This increased urinary output of Be^7 when administered as the citrate is to be expected from the increased excretion noted in the first experiment after early sodium citrate treatment. This was accompanied by a

marked reduction in the Be^7 content of the liver and other viscera. The skeleton, however, contained about twice as much of the Be^7 as it did following Be^7 chloride. Therefore, the difference in carcass content between the animals of the two experiments was small. Fecal excretion was low.

TABLE III. EFFECT OF CALCIUM GLUCONATE AND THE CITRATES OF SODIUM, ALUMINUM, ZIRCONIUM, AND THORIUM ON THE DISTRIBUTION AND EXCRETION OF CARRIER-FREE Be^7 INJECTED AS A CITRATE SALT

	UNTREATED CONTROLS (3 RATS) (% * PER ORGAN)	SODIUM CITRATE (2 RATS) (% PER ORGAN)	CALCIUM GLUCONATE (1 RAT) (% PER ORGAN)	ALUMINUM CITRATE (2 RATS) (% PER ORGAN)	ZIRCONIUM CITRATE (2 RATS) (% PER ORGAN)	THORIUM CITRATE (2 RATS) (% PER ORGAN)
Carcass	35.1 ± 1.5†	34.3 ± 1.3	36.2	31.6 ± 1.4	34.5 ± 0.3	35.6 ± 1
Femur	1.82 ± 0.09	1.86 ± 0.1	1.78	1.52 ± 0.12	1.84 ± 0.2	1.85 ± 0.13
Liver	0.76 ± 0.05	0.85 ± 0.15	0.86	1.95 ± 0.09	1.20 ± 0.1	1.14 ± 0
Pancreas	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Spleen	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mesenteric lymph node	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Kidneys	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Lungs	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Blood	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Urine, 1 day	41.6 ± 2.9	36.6 ± 6	47.1	47.8 ± 4	45.8 ± 0.6	41.1 ± 2.9
2 day	3.4 ± 1.0	2.6 ± 0.4	1.4	2.0 ± 0.2	1.4 ± 0.1	3.0 ± 0.8
3, 4, 5 day	1.8 ± 0.06	1.5 ± 0.2	1.2	1.5 ± 0.04	1.6 ± 0	0.89 ± 0.36
6 day	0.54 ± 0.3	0.37 ± 0.07	0.23	0.63 ± 0.02	0.3 ± 0.1	0.28 ± 0.05
7, 8 day	0.43 ± 0.06	0.37 ± 0.02	0.44	0.80 ± 0.12	0.4 ± 0	0.26 ± 0.05
Total urine	47.8	41.4	50.4	52.7	49.5	45.5
Total feces	3.5 ± 1.0	ca. 7	2.3	3.6 ± 1.9	2.4 ± 0.1	2.5 ± 0.2
Recovery minimum	89.0	85.5	91.5	91.4	89.4	87.6

*Per cent refers to per cent of the injected dose of Be^7.

†Each tabulated value is an average obtained from the number of animals indicated above. It is followed by the average deviation.

No effect on the excretion of Be^7 when given as the citrate was observed after administration of salts of Ca, Na, Al, Zr, and Th (Table III). Distribution of Be^7 was also unaffected, with the possible exception of a slightly increased liver content following the injection of the last three elements.

Intravenous Injection in Dog: Longer term data on excretion of Be^7 were obtained following the injection of Be^7 into an adult male dog. The dog weighed 10 kilograms at injection and 13.9 kilograms at sacrifice 128 days later. It was injected intravenously with 25 c.c. of a 1 per cent sodium citrate solution containing carrier-free Be^7 at a pH of 5.7. This was prepared by adding sodium citrate to a solution of Be^7 in HCl and adjusting the pH with 1N NaOH. The dog received about 0.9 mc. of Be^7 or 125,000 c.p.m. At sacrifice, radioactive decay had reduced this to about 28,000 c.p.m. From the twenty-seventh to the one hundred twenty-sixth day intravenous injections of either sodium, zirconium, or aluminum citrate were given at intervals (Fig. 3).

About two months after injection the rate of excretion of Be^7 was roughly constant at about 0.05 per cent of the injected dose per day in the urine and about 0.005 per cent in the feces (Fig. 3). The administration of citrates had no significant effect on excretion, although a transitory increase in fecal output followed at least one administration of sodium citrate. At sacrifice the bone and liver had the highest concentrations of Be^7 (Table IV). Small but definite

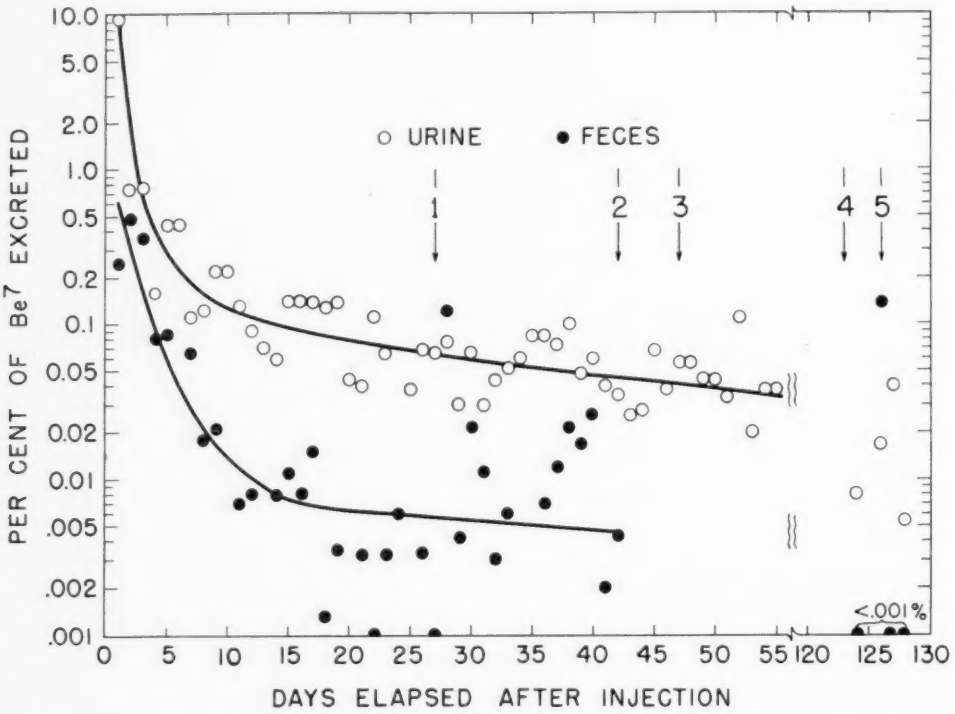

Fig. 3.—Urinary and fecal excretion by the dog of carrier-free Be^7 following intravenous injection as the citrate salt and the effect of various treatments on the excretion. The arrows represent the following intravenous treatments: *1*, sodium citrate, 625 mg.; *2*, zirconium citrate, 260 mg. as Zr; *3*, aluminum citrate, 25 mg. as Al; *4*, sodium citrate, 525 mg.; *5*, sodium citrate, 625 milligrams.

TABLE IV. DISTRIBUTION OF Be^7 IN THE TISSUES OF A DOG 128 DAYS AFTER INTRAVENOUS INJECTION AS THE CITRATE SALT

TISSUE	FRESH WEIGHT ANALYZED (GM.)	AMOUNT OF Be^7 IN TISSUE	
		% PER ORGAN	% PER GRAM
Femur, complete	52.3	0.94	0.018
Femur, without marrow	45.3	0.96	0.021
Skull	22.7	- -	0.013
Bone marrow	0.65	0*	- -
Liver	109	(7.4)†	0.021
Spleen	68.0	0.11	0.0016
Kidney, cortex	55.0	0.10	0.0018
Kidney, medulla	10.7	0.007	0.0007
Lungs	96.8	0.10	0.0010
Brain	84.1	0.005	0.0006
Blood	- -	<0.6	- -
Testes	28.0	0.008	0.0003
Heart	98.2	0	- -
Pancreas	33.1	0	- -
Mesenteric lymph node	4.2	0	- -
Mediastinal and bronchial nodes	1.06	0	- -
Adrenals	1.9	0	- -
Thyroids and parathyroid	1.34	0	- -
Pituitary	0.084	0	- -

*A zero indicates that no significant activity above that of the background of the counter was detectable in the sample.

†Calculated by assuming that the total liver weighed 350 grams.

amounts, however, were found in the spleen, kidney cortex, and lungs. The femur retained one and one-half times as much Be^7 per gram as did the flat skull bones. The kidney cortex retained about two and one-half times as much Be^7 per gram as did the medullary regions.

DISCUSSION

Origin of Beryllium-Induced Granuloma and Possibilities for Treatment.—Ionized beryllium is a protoplasmic poison. The amount of beryllium which can cause histologically observable tissue damage is less than 0.01 μg per gram of tissue. Insoluble beryllium in the form of the metal, oxide, or silicate may reside in the lungs for a period of years. Eventually each particle becomes surrounded by a small nodule of damaged tissue because of repeated insults (i.e., oligodynamic action) by traces of solubilized beryllium which diffuse from the periphery of each individual particle. The time required for the production of the beryllium granuloma is a function of the solubility of the beryllium compound. The solubility is dependent upon the composition of the body fluids and the surface properties of the beryllium particles. For example, the very low solubility of BeO, which is of the order of 45×10^{-6} μg per cubic centimeter,* means that tissue damage may be delayed for several years. On the other hand, fairly soluble or highly insoluble forms of beryllium may not cause damage; the former because the sustained conditions for repeated insult do not exist, and the latter because the amount of ionized beryllium produced is too small to induce damage.

The successful treatment of beryllium granuloma would then involve the removal or the immobilization of the ionized beryllium. The former might be accomplished by the use of complexing agents such as the citrates which form a soluble and diffusible complex ion with beryllium[10] and other metals. Citrates have therefore been used in the treatment of experimental uranium and lead poisoning[11, 12] and to reduce the inhibitory effect of Be on alkaline phosphatase.[13] Since citrate injection twenty-four hours after Be administration has no effect on Be metabolism (because the retained Be has been either bound to protein[14] or converted to insoluble forms), it is unlikely that the lung or body content of the insoluble forms of Be can be reduced significantly by citrate treatment. Furthermore, larger amounts of citrates are required to dissolve even the hydroxides of elements such as Be[10] and Th[15, 16] after they have formed than are necessary to prevent their precipitation. The citrates may even enhance the toxicity of Be by increasing the local concentrations of ionized Be.†

As far as the second alternative for treatment, immobilization, is concerned, it is possible that the amount of ionized Be around the Be particle might be reduced or eliminated by reaction with a reagent which forms an insoluble compound or lake with ionized Be.‡

*Depending on previous treatment, samples of BeO vary greatly in their solubility. The relationship between the solubility (surface properties) and previous treatment of the BeO can be predicted qualitatively.

†We have found that the administration of sodium citrate to rats given acute lethal doses of beryllium sulfate intraperitoneally decreased the survival time even though the twenty-four hour urinary excretion was increased from 0.8 per cent to 6 per cent of the injected dose.

‡We have observed that the intraperitoneal administration of manganous gluconate to rats given acute lethal doses of beryllium sulfate intraperitoneally increased their survival time. The mechanism here may be the adsorption of a fraction of the beryllium by the manganous hydroxide which forms at physiologic pH.

Beryllium Metabolism.—The gross metabolism of Be and other elements can be interpreted by considering the solution chemistry of cations and the metabolism of colloids. Concepts previously presented[15, 17] clarify the metabolic behavior of beryllium as reported in this paper. Some of the pertinent facts regarding the chemistry of Be are: (a) Be is precipitated as an insoluble hydroxide from dilute inorganic solutions at a pH of about 5.7[18]; (b) colloids of large size disappear rapidly ($t_{½}$ = 30 sec. to 1 min.) from the blood stream and are deposited mainly in the liver and spleen[9]; (c) simple polyvalent cations also leave rapidly and are deposited principally in the bones; (d) with decreasing size the colloidal particles disappear more slowly from the blood stream and are deposited primarily in the bone marrow and spleen[9]; (e) the size of colloidal aggregates increases as the amount of carrier associated with the tracer element is increased.[19]

A soluble Be compound injected into the blood stream tends to become converted to the nondiffusible hydroxide form because of the elevated pH and to become bound to protein.[14] Complexing anions, e.g. citrate, if administered before the conversion of the major part of the Be to insoluble or protein-bound states, form easily diffusible compounds with the Be in the blood and soft tissues. The complex ions are rapidly excreted, but the rate of deposition into bone is simultaneously increased. This probably explains the more rapid excretion, lower soft tissue retention, and higher skeletal content of Be^7 when administered as the citrate than when given as the chloride (Tables I and III). It also explains the effect of sodium citrate administration on the metabolism of Be^7 chloride.

The increased excretion of Be^7, administered as the chloride, following zirconium treatment is at the expense of the skeleton (Table I). The mechanism probably involves an adsorption of Be on the colloidal aggregates formed by zirconium in the blood. Previous studies on the effect of zirconium on plutonium and yttrium metabolism[20] have shown that the early injection of Zr compounds immediately removes nearly all of the radioelement from the circulation, thereby preventing further bone deposition.

SUMMARY

Following intraperitoneal injection of Be^7Cl_2 into rats, urinary excretion of Be^7 was about 13.6 per cent of the injected dose during the first twenty-four hours and subsequently fell to about 0.5 per cent each day. After intraperitoneal injection of Be^7 as the citrate complex, however, 40 per cent of the dose was found in the first day's urine. The femur contained twice as much Be^7, while the liver, other viscera, and probably the muscle contained much less Be^7 than after Be^7 chloride injection.

Sodium or zirconium citrate injected intraperitoneally two hours after the injection of the Be^7Cl_2 increased the urinary excretion of Be^7 from 13.6 per cent to 32 and 36 per cent respectively during the next twenty-four hours. After sodium citrate administration the Be^7 content of the liver, spleen, and kidney was reduced significantly; while after zirconium citrate administration the skeletal

content was lower. Neither citrate given six days later affected Be^7 metabolism. Calcium gluconate or the citrates of Na, Al, Zr, and Th given to the Be^7 citrate-injected rats had no effect on the distribution or excretion of Be.

The distribution of Be^7 injected as the chloride differed markedly with the route of administration. After intraperitoneal injection into rats the pancreas contained 15 per cent of the dose. After intravenous injection there was no Be^7 in the pancreas, but much more in the skeleton, liver, and spleen.

Two months after intravenous injection of Be^7 citrate the urinary excretion of Be^7 by an adult dog was about 0.05 per cent of the injected dose per day, the fecal excretion about 0.005 per cent. Administration of the citrates of Na, Zr, or Al twenty-seven or more days after Be injection did not affect Be^7 excretion. Most of retained Be^7 was in the skeleton, particularly the long bones, 128 days after injection.

The results obtained were interpreted by a consideration of the chemical and colloidal properties of beryllium.

The production of beryllium-induced granulomas and possibilities for their treatment are discussed.

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HYPOURICEMIA: WITH EVIDENCE FOR TUBULAR ELIMINATION OF URIC ACID

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SINCE the reported values of uric acid clearance are small compared with the rate of glomerular filtration, it is generally assumed that the uric acid excretion through the kidney is exclusively a filtration reabsorption mechanism.

In the course of an investigation of a larger group of subjects, with the purpose of revising the normal plasma uric acid values in man, a person was encountered who on repeated examinations showed an excessively low concentration of plasma uric acid (0.2 to 0.6 mg. per cent) and at the same time a quite normal daily output of this compound in the urine. These findings led us to a study of his uric acid excretion.

In view of the very low plasma uric acid values it was further decided to determine the concentration of the uric acid oxypurine precursors in his plasma and to perform similar plasma studies on his close relatives and, for comparison, on a group of normal controls. The results of these plasma analyses and of the uric acid excretion studies are presented in this paper.

The subject was a 28-year-old white man who had never suffered from any serious disease and who enjoyed perfect health at the time he volunteered for the study. A physical examination revealed no abnormalities.

EXPERIMENTAL

The plasma uric acid analyses were performed by enzymatic differential spectrophotometry as described in detail by one of the authors.[1] In this procedure 0.2685 ml. of plasma is diluted with glycine buffer of pH 9.4 to a volume of 10 ml. and the extinction of the diluted sample contained in a silica cell is measured at 292.5 mμ in a Beckman spectrophotometer before, during, and after the action of a highly purified sample of uricase. The standard deviation of the procedure with plasma of normal uric acid concentration is less than 1.5 per cent. In order to secure a similar accuracy in the examination of plasma from the individual with hypouricemia, besides the usual plasma dilution (approximately 1:40), dilutions of 1:8 were prepared as well by perchloric acid protein precipitation as by ultrafiltration of the plasma through a cellophane membrane. The uric acid values obtained by these three procedures of enzymatic spectrophotometry agreed closely.

In order to rule out the possibility that the low uric acid values observed were conditioned by the presence in the plasma of a factor inhibiting the action of uricase, uric acid was added to the subject's plasma in an amount corresponding to a plasma concentration of 4 mg. per cent; this added uric acid was quantitatively recovered by the spectrophotometric uricase technique. For further substantiation of the validity of the low plasma uric acid concentration, parallel determinations were made by the enzymatic technique and the colorimetric reduction method of Brown.[2] Practically identical results were obtained in the two procedures.

The uric acid determinations in urine were performed on samples diluted with glycine buffer in the same manner as used in the analysis of normal plasma.[1]

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The assay of uric acid oxypurine precursors in the plasma was made by measuring uric acid before and after the action of xanthine oxidase (Ball's enzyme).[3] The determination was made by adding a sample of this enzyme* to undiluted plasma contained in a tube which was immersed in a water bath at 37° C. for two to four hours. The uric acid formed was subsequently measured in the Beckman spectrophotometer by the uricase method as described. It was ascertained that xanthine dissolved in glycine buffer could be determined quantitatively by this procedure and that xanthine added to plasma could be recovered under the conditions of the technique. Duplicate analyses usually agreed within 5 per cent.

Determination of inulin in plasma and urine was carried out as described by Rolf, Surtshin, and White.[4] For the clearance determination the inulin was injected subcutaneously in the axillary regions.

RESULTS

The plasma values of uric acid and of uric acid oxypurine precursors observed in the person with hypouricemia on four different occasions are presented in Table I which also contains the results found in analyses of blood samples from his relatives and from eight normal controls. The range of plasma uric acid in the subject with hypouricemia is seen to be from 0.2 to 0.6 mg. per cent, whereas the uric acid values found in his relatives are within normal limits.

TABLE I. THE PLASMA CONCENTRATION OF URIC ACID AND OF OXYPURINE PRECURSORS OF URIC ACID IN THE PERSON WITH HYPOURICEMIA, HIS RELATIVES, AND NORMAL CONTROLS

SUBJECT	SEX	AGE	PLASMA URIC ACID (MG. %)	PLASMA OXYPURINE PRECURSORS OF URIC ACID (MG. %)	COMMENTS
J. F.	M	28	0.3	5.2	Oct. 8, 1949
			0.2		Nov. 23, 1949
			0.6	5.1	Dec. 8, 1949
			0.3		Dec. 18, 1949
J. F.	M	64	5.7	4.9	Father
J. F.	M	7	2.5	4.5	Son
W. F.	M	21	5.7		Brother
C. B.	F	35	3.1	1.6	Sister
R. S.	F	63	3.8		Father's sister
L. B.	F	53	4.5	1.7	Mother's sister
E. K.	M	44	4.1	0.5	Control
M. C.	F	36	3.6	2.1	Control
E. P.	M	41	5.9	0.5	Control
M. M.	F	24	3.2	1.1	Control
F. S.	M	36	5.5	1.4	Control
M. O.	F	62	2.8	0.2	Control
A. G.	F	70	4.0	0.8	Control
K. M.	F	66	3.4	0.9	Control

The concentration of uric acid oxypurine precursors was high both in the plasma of the individual (5.1 to 5.2 mg. per cent) and in the plasma samples obtained from his father (4.9 mg. per cent) and son (5.4 mg. per cent), but the values observed in his mother's sister (1.7 mg. per cent) and his sister (1.6 mg. per cent) were within normal limits. In the control group the concentration of uric acid oxypurine precursors varied from 0.2 to 2.1 mg. per cent (mean, 1.0 mg. per cent). This average value is higher than that recently reported by Wolfson and co-workers[5] using crude enzyme preparations and reduction colorimetry of uric acid.

*The xanthine oxidase was kindly supplied by Professor Herman Kalckar, Cytophysiological Institute, University of Copenhagen, Denmark.

Determination of the urinary excretion of uric acid was performed on a twenty-four hour sample which showed an output of 690 milligrams. Uric acid clearance tests were subsequently performed on two different occasions; in connection with one of the tests a simultaneous inulin clearance determination was made. The results of the clearance measurements are given in Table II which shows uric acid clearance values ranging from 162 to 284 ml. per minute. The values must be characterized as excessively high, when compared with the normal uric acid clearance values of 10 to 35 ml. per minute. A comparison between the simultaneously determined uric acid and inulin excretion figures reveals uric acid clearance values definitely above those of inulin. The average difference between the two sets of clearance values is 46 per cent and the difference between the lowest uric acid clearance and the highest inulin clearance is 28 per cent.

TABLE II. URIC ACID AND INULIN CLEARANCE VALUES OBSERVED IN THE PERSON WITH HYPOURICEMIA

EXCRETION PERIOD (MIN.)	URINE VOLUME (ML.)	DIURESIS PER MIN. (D)	CONCENTRATION (MG. %) OF URIC ACID IN URINE (U)	CONCENTRATION (MG. %) OF URIC ACID IN PLASMA (P)	CONCENTRATION (MG. %) OF INULIN IN URINE (U)	CONCENTRATION (MG. %) OF INULIN IN PLASMA (P)	CLEARANCE $\frac{D\ U}{P}$ URIC ACID	CLEARANCE $\frac{D\ U}{P}$ INULIN
24	305	12.6	4.6	0.3	92.2	9.03	193	127
37	474	12.8	3.8	0.3	84.6	9.77	162	113
29	406	14.0	3.7	0.3	84.4	9.77	170	119
85	92	1.08	50.3	0.20			272	
59	151	2.56	23.3	0.21			284	

DISCUSSION

The findings reported show that in the present case uric acid is excreted by tubular secretion or formed in the kidney.

In spite of the fact that the plasma concentration of oxypurine is considerably higher than usually found in man, it is, however, not probable that uric acid should be formed by a *direct* oxidation of the precursors found in his blood by the action of a kidney xanthine oxidase. All attempts at disclosing the presence of such an enzyme in the human kidney (including some by us) have as yet failed.

SUMMARY

A case of hypouricemia (plasma uric acid 0.2 to 0.6 mg. per cent) in a 28-year-old white man is described. The plasma concentration of oxypurine uric acid precursors was considerably increased (5.1 to 5.2 mg. per cent against a normal average of 1.0 mg. per cent).

The daily excretion of uric acid was within normal limits. The uric acid clearance was about ten times higher than the normal average and exceeded the simultaneously determined inulin clearance by about 50 per cent.

The findings show that in this case a renal secretion or formation of uric acid takes place.

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VARIATIONS IN THE SERUM POLYSACCHARIDE AND SEDIMENTATION RATE IN CLINIC PATIENTS

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IT WAS reported by Seibert and co-workers[1, 2] that an increase in a polysaccharide constituent occurred in the sera of patients with tuberculosis and various types of carcinoma. The extent of the increase paralleled the extent of the disease and appeared to accompany an increase of the alpha$_2$ globulin fraction of the serum as determined electrophoretically. It was suggested that this serum constituent increases in diseases characterized by tissue destruction or acute inflammation.

The present study was undertaken to determine the degree of specificity of the test on sera from unselected patients visiting the clinics of the Henry Phipps Institute for diagnosis or follow-up examination.

While this study was in progress, Israel, Webster, and Maher[3] published a similar study on 102 ward patients with a variety of diseases other than tuberculosis.

DIAGNOSTIC PROCEDURES

The sera were examined and classified according to a diagnosis reached by x-ray examination of the chest, clinical examination, and laboratory studies.

A group hereafter designated as normal consisted of patients who had no abnormal chest x-ray finding, negative clinical examination, and normal laboratory findings. The remainder of the patients were grouped according to diagnosis reached after x-ray examination of the chest, clinical examination, laboratory studies, and history, as shown in Table I. The mean values, the ranges, the standard deviations, the probable error of the means, and the significance factors for the deviation from the normal means for the polysaccharide (T.A.) results were calculated by a method which will be described.[1] The mean values and ranges for the sedimentation rates were also reported.

LABORATORY METHODS

The polysaccharide in the serum was determined by the T.A. method described by Seibert, Pfaff, and Seibert,[1] based on the reactions between tryptophane (T), perchloric acid (A), and certain components of sera, probably polysaccharide in nature. The results are recorded as Klett photocolorimetric readings.

The sedimentation rate was determined by the method of Cutler.[4] The results are recorded for convenience in the form of fractions, the numerator denoting the maximum sedimentation rate in millimeters in any five-minute period during the first half hour, and the denominator, the reading after one hour. A rate not exceeding 1 mm. in any five-minute period is considered normal. Values above 1 mm. are counted as abnormal. The numerator is considered most significant.

Statistical analyses were made in the manner previously given.[2] A significance factor, as described therein, of five or more indicates a significant deviation from the normal mean values.

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RESULTS

The results are shown in Table I.

TABLE I. COMPARISON OF THE T.A. TEST AND SEDIMENTATION RATE IN CLINIC PATIENTS

TOTAL NUMBER OF PATIENTS: 495	ANALYSIS OF SERA BY T.A. METHOD						SEDIMENTATION RATE		
TYPE OF CASE	NUMBER	MEAN	RANGE	STANDARD DEVIATION	PROBABLE ERROR OF THE MEAN	SIGNIFICANCE FACTOR	MEAN	RANGE	
Normal chest x-ray No symptoms	111	55	33-84	9.8	0.6		0.9/11	0.9/0.5	7/26
Tuberculosis Active: Minimal	10	70	48-115	17.5	3.7	—	1.5/11	0/3	6/27
Moderately advanced	46	84	60-136	19.4	2.8	10.4	2.8/16	0.5/1.5	11/28
Far advanced	25	107	68-155	22.2	4.4	11.8	3.7/21	1/5	10/32
Questionable significance: Minimal	45	60	34-102	14.6	1.5	—	1.2/8	0/1	5/24
Moderately advanced	30	63	36-102	14.3	1.8	—	1.8/9	0/2	2.5/21
Apparently arrested: Minimal	26	62	49-75	7.0	1.4	5.0	1.4/9.5	0/2	7/26
Moderately advanced	10	58	47-63	4.8	1.0	—	1.5/6.8	1.0/4.0	1.5/8
Retrogressive	9	58	49-70	8.8	2.0	—	1/7	0/0.5	1.5/13
Primary type (healed)	20	60	34-80	11.0	1.5	—	1.6/9	0/1.5	7/24
Diseases other than tuberculosis									
Sarcoidosis	7	85	63-106	15.5	4.0	7.5	3.4/19	1/16	8/26
Nontuberculous basal lesions	72	62	34-95	11.1	0.9	7.7	1/7.9	0/1	6/22
Cardiac abnormalities	25	75	54-134	20.6	2.8	7.2	2.7/15	0/0.5	8/25
Other illnesses with normal chest x-ray	59	72	51-104	10.2	0.9	16.0	2.3/13.5	0.5/3	6/26

—, Represents a factor less than 5 or no statistical significance

DISCUSSION

Within the 495, a normal group, 111 in number, who had normal chest x-ray films, no past medical history of serious illness, and no symptoms or complaints, showed an average T.A. value of 55. Included in this group were thirteen patients who gave a history of previous contact with tuberculosis. The average sedimentation rate was 0.9/11.

Tuberculous patients were grouped into active cases, cases of questionable clinical significance, and cases in which the disease was apparently arrested. Ten cases of minimal active tuberculosis showed an average T.A. value of 70,

which is above the normal of 55. The significance factor, however, was only 4.1, which indicated that little significance can be attached to the result. The number of patients apparently was too small to be clinically significant. Seibert, Pfaff, and Seibert[1] also found a moderate elevation in the T.A. value in their twenty-two cases of minimal active tuberculosis. The average T.A. value for their group was 72, and the significance factor was 4.9. The moderately advanced and far advanced cases of active tuberculosis showed a definite increase in the T.A. values, as well as in the sedimentation rates. The significance factors for both groups were markedly elevated. Whether or not the fact that most of these patients were ambulatory and not under favorable home conditions had an effect on the T.A. value is a matter for future investigation.

One patient with minimal active tuberculosis showed a low T.A. value of 48 and normal sedimentation rate of 1/3. This patient, a white woman, aged 32, had no complaints and apparently felt well. She visited our clinic for regular routine checkups. She was delivered of a normal child three months before the tests here recorded. The sputum had been negative for four years. X-ray examination of the chest had shown no essential change within that period. This patient might have been classified as questionably active or apparently arrested; however, there was no evidence of fibrosis or healing of the minimal lesion, and she was thus still classified as active.

Three patients with moderately advanced active tuberculosis had a relatively low T.A. value. One, a Negro woman, aged 29, felt well and had no definite complaints or symptoms. The sputum was negative; the last positive sputum was twenty-two months previously. X-ray examination of the chest, however, showed no evidence of definite healing, and she was therefore still classified as active. The T.A. value in this patient was 60, and the sedimentation rate was 1/3. Another patient, a Negro woman, aged 28, felt well and had no complaints. Sputum examinations and laryngeal swab were negative for acid-fast bacilli. X-ray examination of the chest showed some calcium deposits; however, the appearance in general suggested that the disease was still active. The T.A. value was 60 and the sedimentation rate was elevated, viz., 5/27. The third patient, a white man, aged 25, felt well and had been working six hours daily for the past year. He received pneumothorax treatment from 1943 until 1945. Concentrated smears of the sputum were negative for acid-fast bacilli. X-ray examination of the chest had shown no definite change in twenty-eight months; however, the appearance was still that of active disease. His T.A. value was 49; the sedimentation rate was 1/3.

The group with tuberculosis of questionable clinical significance, both minimal and moderately advanced, showed no significant deviations from the normal T.A. value and normal sedimentation rate. The significance factors were below five. One questionable minimal case showed a marked elevation of the T.A. value and sedimentation rate. The patient, a Negro woman, aged 63, complained of weight loss for the past few months, grippe with fever, and severe shoulder pains, for which she was being treated by a private physician. Physical examination was essentially negative. Sputum studies were negative. X-ray examination of the chest showed minimal disease which was of questionable

activity. The T.A. value was 102 and the sedimentation rate 5/24. Thus both values were markedly elevated. It was quite possible that superimposed nontuberculous infection influenced both figures.

Twenty-six patients with apparently arrested tuberculosis of minimal extent had an average T.A. value of 62. The significance factor, however, was 5.0, which indicates that some significance may be attached to this group. Thirteen patients of this group, however, had various complaints ranging from slight colds with cough to weight loss and grippe with fever, hoarseness, anemia, and malnutrition. One of these patients gave a history of recent operation for tumor of the uterus. It can be surmised that superimposed abnormalities influenced the T.A. value. The sedimentation rate for this group was only slightly elevated.

Retrogressive tuberculosis and primary type healed tuberculosis showed no significant deviations from the normal T.A. values and sedimentation rates.

Cases of sarcoidosis all showed an increase in the T.A. value, as was also true of the sedimentation rate. The identity of these cases as sarcoid disease was proved by biospsy.

The group of patients diagnosed as suffering from nontuberculous basal lesions, as mentioned previously, were diagnosed by x-ray examination of the chest and clinical examination. Most of these patients could be included in a group under the term "chronic bronchitis." The usual x-ray findings consisted of increased markings at the bases mesially. The symptoms consisted of chronic cough with or without expectoration. The average T.A. value for this group was 62; the mean sedimentation rate was 1/7.9 which was within normal limits. However, the significance factor was 7.7, which indicated that significance must be attached to the group as a whole.

The group with cardiac abnormalities consisted of twenty-five patients. These patients were diagnosed by x-ray examination of the chest and clinical examination. No cardiographic studies were made. The average T.A. value for this group was 75; the mean sedimentation rate was 2.7/17.5. Thus both values were elevated. The significance factor was 72, which indicates that this group showed significant abnormality. The highest T.A. values were found in cases of pericardial effusion, cardiac enlargement with pulmonary congestion, and cardiac enlargement with hypertension. Two patients with cardiac enlargement with hypertension showed an average T.A. value of 79. The mean sedimentation rate was 2.2/17.

Three patients of this group had T.A. values of over 100. One of these, a white man, aged 66, complained of chronic cough, stating that he had had cardiac trouble for many years. Physical examination revealed a systolic murmur which was not transmitted. There were occasional moist râles at both bases, and the blood pressure was 158/94. X-ray examination of the chest showed an enlarged heart, dilated aorta, pulmonary congestion, and patchy emphysema. The T.A. value was 114 and the sedimentation rate was 3/19. The second of the three patients, a white man aged 52, complained of weight loss, chest pains, colds, and cough with expectoration for the past two years. There was a history of pneumonia four years previously. Physical examination showed

cardiac hypertrophy and hypertension (188/100). Laboratory studies showed a moderate anemia. X-ray examination of the chest revealed cardiac hypertrophy, dilated aorta, and thickened pleura. The T.A. value was 134; the sedimentation rate was 6/28. The third patient, a Negro man, aged 63, complained of chronic cough with expectoration, dyspnea, and weight loss of ten pounds in three months. Physical examination revealed moderate hypertension (168/92), with basal râles. Laboratory examination showed mild anemia. X-ray examination of the chest showed a dilated aorta and a chronic nontuberculous basal lesion. This patient had a T.A. value of 105; the sedimentation rate was 2/22.

Six patients of the group of twenty-five cardiac cases had positive Kahn tests. Their average T.A. value was 70 and the average sedimentation rates were within normal limits.

Ten cases of dilated aorta were diagnosed by chest x-ray examination. The average T.A. value was 66; the mean sedimentation rate was 1.1/7.9. The significance factor for the T.A. value was 5.8, which indicated some significance.

Fifty-nine miscellaneous cases of other illnesses, with normal chest x-ray films, showed an average T.A. value of 72. The mean sedimentation rate was 2.3/13.5. The significance factor was 16.0, which is highly significant. The symptoms of the group were varied and numerous, ranging from mild to severe. It is thus evident that illnesses other than those listed influence the T.A. value and sedimentation rate. This finding corroborates the report of Israel, Webster, and Maher.[3]

Apparently active disease, whether inflammatory or degenerative, may alter the chemical composition of the blood serum, with an increase in the polysaccharide content. The sedimentation rate in general varies in a manner corresponding to the polysaccharide content.

No significant variation with respect to sex or color was discovered.

SUMMARY

A comparative study was made of the concentration of a polysaccharide constitutent of the serum (T.A. value) and erythrocyte sedimentation rate in 495 patients attending an outpatient clinic.

Patients with moderately advanced and far advanced active tuberculosis showed a definite increase in the T.A. value, as well as in the sedimentation rate.

Patients with retrogressive tuberculosis and primary type healed tuberculosis showed no significant deviations from the mean normal T.A. value and sedimentation rate.

All patients with sarcoidosis showed an increase in the T.A. value and sedimentation rate.

Patients with nontuberculous basal lesions, as a rule due to chronic bronchitis, showed an increase in T.A. value of slight significance but a normal sedimentation rate.

Both the T.A. value and sedimentation rate were elevated in the group of patients with cardiac abnormality.

Miscellaneous illnesses were associated with elevated T.A. values and sedimentation rates.

No significant variation with respect to sex or color was discovered.

Acknowledgment is made to Dr. E. R. Long and Dr. F. B. Seibert for their valuable assistance, advice, and criticism in the preparation of the manuscript.

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COMPARISON OF THE IN VITRO ACTIVITY OF NISULFAZOLE WITH THAT OF OTHER SULFONAMIDES

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LITTLE attention has been given previously to the nitro derivatives of the sulfonamides. Several groups of investigators have demonstrated that these compounds possess bactericidal activity in vivo and in vitro.[1, 2, 3] Rosenthal[4] has shown that the nitro derivatives of sulfanilamide, sulfathiazole, sulfapyridine, and 4-4′-diamino diphenylsulfone exert a much greater inhibitory action against Clostridia than do the more commonly used sulfonamides but are no more effective against hemolytic streptococci. Because of the promising results which have been obtained clinically in the treatment of lower intestinal infections, including ulcerative colitis,[5] and because of the apparent low toxicity of 2-(*p*-nitrobenzenesulfonamido)-thiazole,* we decided to study the in vitro action of this drug upon a variety of enteric pathogens and other organisms, and to compare its activity with that of several other sulfonamides.†

Brewer[6] found that Sulfasuxidine was active in vitro against *Eberthella dysenteriae* Sonne, whereas sulfaguanidine showed no inhibitory action against the same organism. Kirby and Rantz[7] were unable to demonstrate any appreciable activity in vitro on the part of Sulfasuxidine. Only a slight antibacterial action of Sulfasuxidine against *Escherichia coli* was found by Poth and coworkers.[8] The results of Schweinberg and Yetwin[9] indicated that Sulfathalidine had a greater antibacterial action than sulfathiazole against *Eberthella typhi* and *E. dysenteriae* Flexner. They also found that sulfathiazole was the more effective of the two drugs against *Esch. coli*, *E. dysenteriae* Shiga and *E. dysenteriae* Hiss.

Since Nisulfazole is reduced in the gastrointestinal tract with the liberation of free sulfathiazole, experiments were also set up to determine whether the in vitro activity of Nisulfazole is due solely to free sulfathiazole or is due, in part at least, to the nitro compound itself or to some intermediate reduction product. Florestano and Kennedy[10] have shown that the in vitro activity of succinylsulfathiazole and phthalylsulfathiazole is entirely dependent upon the presence of free sulfathiazole, which is liberated upon hydrolysis of these compounds.

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*2-(*p*-Nitrobenzenesulfonamido)-thiazole will be referred to as Nisulfazole.

†Nisulfazole, Nisulfadine, and Sulfasuxidine were obtained through the courtesy of George A. Breon & Co., Kansas City, Mo. Sulfathalidine was supplied through the courtesy of Sharp & Dohme, Inc., Philadelphia, Pa.

METHODS

A. Drug Medium Preparation.—A number of methods have been described for testing the in vitro bacteriostatic and bactericidal properties of sulfonamides.[11-15] Since most sulfonamides are relatively insoluble in water, stock solutions of all the drugs, except sulfaguanidine, were prepared by dissolving 1.0 Gm. in 50 ml. of N/10 sodium hydroxide. Sulfaguanidine was dissolved in hot distilled water. Tryptose phosphate broth containing 1, 5, 10, 15, 20, 25, 30, and 35 mg. per cent of the various drugs in solution was then prepared and the pH adjusted to 7.3 to 7.5. Because of the 5 per cent loss in volume that occurs during autoclaving, the total volume for each 100 ml. quantity of drug medium was 105.3 milliliters. Into each tube, 10.4 ml. of drug medium were then pipetted. After autoclaving, each tube contained 9.9 ml. to which 0.1 ml. of bacterial suspension was added.

In addition to the solutions of drugs, suspensions of sulfaguanidine and Nisulfazole in tryptose phosphate broth containing 1 per cent acacia were compared with solutions for effectiveness in inhibiting the growth of three of the organisms (*Bacillus coli, E. typhi,* and *E. dysenteriae* Flexner). This was done since drugs acting in the intestinal tract are partially in solution and partially in suspension. The suspensions were not entirely satisfactory since a small amount of settling occurred. Higher concentrations of acacia resulted in even more settling. Tubes were rotated three times during the twenty-four hour incubation period to minimize the settling effect.

B. Bacterial Culture Preparation.—Twenty-four hour broth cultures of organisms* were compared with a McFarland barium sulfate nephelometer and diluted with physiologic saline so that each milliliter contained 20,000 organisms. One tenth milliliter of this final dilution was inoculated into 9.9 ml. of testing medium and into 9.9 ml. of the same medium containing no drug. Thus each tube contained 2,000 organisms per milliliter at the time of inoculation.

PROCEDURE

A. Study of Antibacterial Acitivity of Nisulfazole.—Series of drug media were first inoculated with a variety of organisms for comparison of the activity of sulfathiazole with Nisulfazole since reduction of Nisulfazole, with the release of free sulfathiazole, occurs in the presence of some bacteria.[5] In addition to this, the following procedure was used to determine whether the action of Nisulfazole is due solely to the free sulfathiazole released or whether the nitro compound itself or an intermediate reduction product also exerts an inhibitory effect upon the growth of bacterial organisms.

Solutions of Nisulfazole were made in distilled water and in tryptose phosphate broth in concentrations ranging from 1 to 35 mg. per cent and tested for free diazotizable substance by the method of Bratton and Marshall.[16] Nisulfazole determinations were also made, using the same procedure, after reduction of Nisulfazole with zinc dust.[17] Aliquot portions of each solution were autoclaved for twenty minutes at 15 pounds pressure and free sulfathiazole and Nisulfazole determinations were made. Similar determinations were made on the tryptose phosphate-Nisulfazole media after twenty-four hours of incubation with bacterial organisms.

B. Comparison of Antibacterial Activity of Nisulfazole With That of Sulfapyridine, Nisulfadine, Sulfaguanidine, Sulfasuxidine, and Sulfathalidine.—Nisulfazole was compared with these other sulfonamides by the simultaneous incubation of series of the various drug media with organisms. Each series was repeated three or more times. The degree of inhibition of growth was determined by comparison with the nephelometer after 24 hours of incubation at 37° C. One milliliter and dilutions thereof were then plated out from each tube which showed little or no evidence of growth and from the control tube to determine the bactericidal concentration of the drug.

The bacteriostatic concentration is the lowest concentration of drug which causes the number of bacteria per milliliter to be less than in the control tube after twenty-four hours

*Most of the organisms used were supplied through the courtesy of the United States Public Health Service.

of incubation. The bactericidal concentration is the lowest concentration of the drug which completely destroys the bacteria introduced so that no colonies develop when 1 ml. of the test medium is plated out after twenty-four hours of incubation with the organisms.

RESULTS

A. Antibacterial Activity of Nisulfazole.—The antibacterial activity of Nisulfazole as compared with sulfathiazole is shown in Figs. 1 and 2. Nisulfazole is far more effective than sulfathiazole against both strains of *B. coli*, *E. dysenteriae* Flexner, *Salmonella enteritidis*, and *Bacillus perfringens* and more effective but to a lesser degree against many other organisms.* This is one

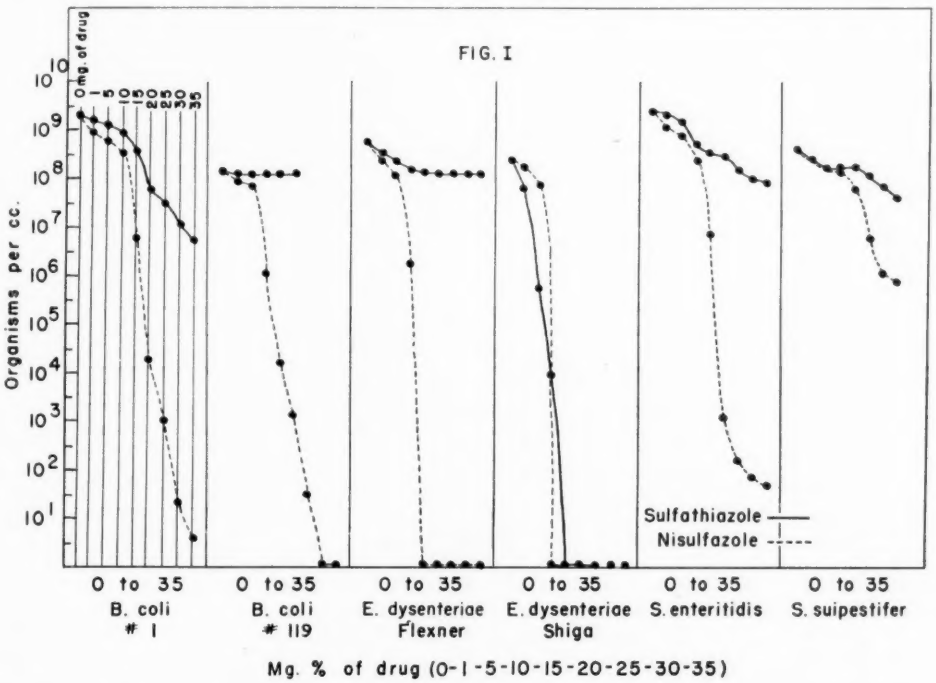


Fig. 1.—Comparative bacteriostatic and bactericidal effects of sulfathiazole and Nisulfazole showing greater inhibition of growth by Nisulfazole.

factor which indicates that the antibacterial action of Nisulfazole is due to something more than the sulfathiazole which is released upon its reduction. Further evidence is given by the analysis of the drug medium. The amount of free sulfathiazole in the Nisulfazole medium before inoculation with organisms is quite small. Even though complete reduction takes place during incubation with some organisms (*B. coli* 1, *Staphylococcus aureus* 1, *Staph. albus* 1, *Staph. citreus*, and β-hemolytic streptococcus 1), the amount of free sulfathiazole

*Sulfathiazole was equally as effective as Nisulfazole in inhibiting the growth of: *Salmonella suipestifer*, *Br. abortus*, *Staph. albus* 1, β-hemolytic streptococcus 1, and β-hemolytic streptococcus c-203.

In the case of the following organisms, sulfathiazole exhibited greater antibacterial activity than did Nisulfazole: *E. typhi* 24, *Salmonella paratyphi* A 39, *Salmonella paratyphi* B 43, *Salmonella morgani* 16-c5, *Salmonella aertrycke* 545, *Aerobacter aerogenes* 527, *Alcaligenes faecalis* 113, *pyocyaneus* 279, *Brucella melitensis* 428, *Friedländer's* bacillus, *Corynebacterium diphtheriae* (Park 8) 385, *Staph. citreus*, β-hemolytic streptococcus 5.

present still does not exceed the quantity of Nisulfazole which was added to the medium. Nor does it exceed the amount of sulfathiazole in the comparable sulfathiazole medium. Yet in the case of all the organisms shown in Figs. 1 and 2, Nisulfazole has produced greater inhibition of growth. Even in several cases in which little or no reduction occurred (*B. coli* 119, *E. dysenteriae* Shiga, *E. dysenteriae* Flexner, and *Staph. aureus* C24), the growth of the organisms

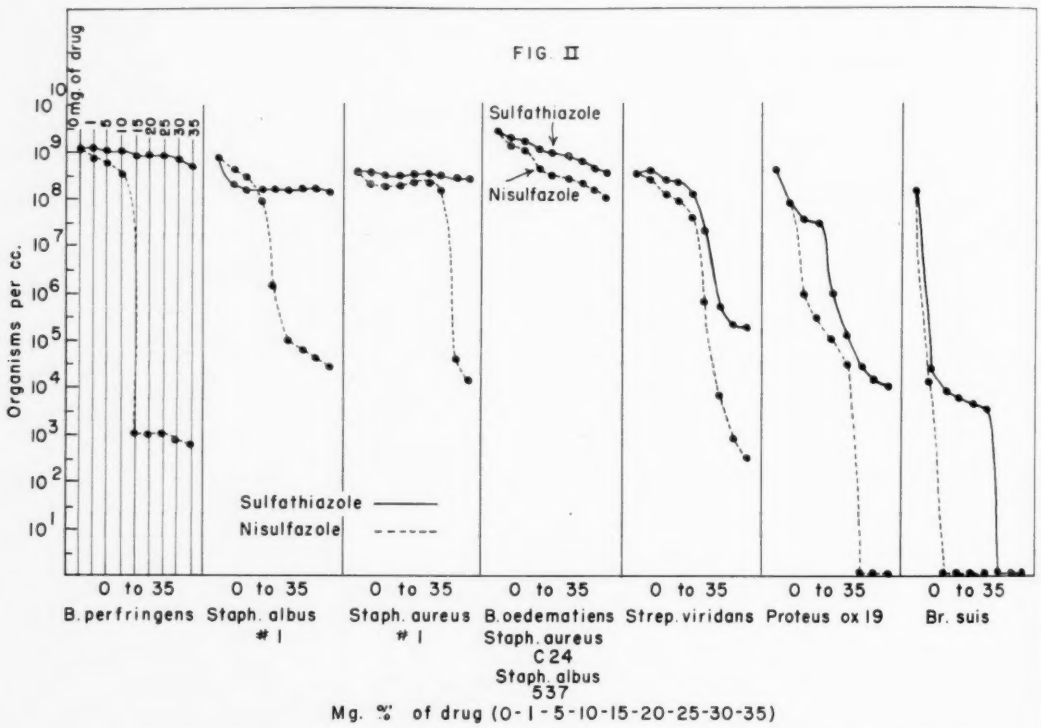


Fig. 2.—Comparative bacteriostatic and bactericidal effects of sulfathiazole and Nisulfazole showing greater inhibition of growth by Nisulfazole.

was more markedly inhibited by Nisulfazole than by sulfathiazole. Since Nisulfazole is even more effective than is the amount of sulfathiazole which it is capable of releasing, we must conclude that the action of Nisulfazole is not entirely due to sulfathiazole but is due, in part at least, to the nitro compound itself or to some intermediate reduction product.

A few other findings in connection with the chemical analysis of the drug media and drug solutions were:

(1) Aqueous solutions of Nisulfazole contained no free diazotizable substance, either before or after autoclaving.

(2) Tryptose phosphate broth solutions of Nisulfazole contained no free sulfathiazole before autoclaving but did afterward in amounts ranging from 0.6 mg. per cent in the 1.0 mg. per cent to 3.8 mg. per cent in the 30 mg. per cent Nisulfazole medium.

(3) Autoclaving destroys a portion of the Nisulfazole or changes it into another compound which does not give a color reaction since:

(a) Eighty per cent (which is the limit of accuracy of the method) of the Nisulfazole could be recovered before autoclaving but only 50 to 80 per cent afterward.

(b) The inhibitory action of Nisulfazole which had not been autoclaved was slightly greater than that of Nisulfazole which had been autoclaved.

B. Comparison of Antibacterial Activity of Nisulfazole With That of Other Sulfonamides.—The results are shown in Figs. 3 and 4. Nisulfazole had a more marked inhibitory effect on the growth of *B. coli*, *S. paratyphi* A, *S. paratyphi* B, *S. enteritidis*, *E. dysenteriae* Flexner, *E. dysenteriae* Shiga, and *Streptococcus viridans* than any of the other sulfonamides tested. It surpasses the others in its action against all the organisms tested with the exception of *Staph. albus*, *Br. abortus*, *E. typhi*, and *Bacillus oedematiens*.

Rather unusual curves were obtained with Sulfathalidine in the presence of *B. coli*, *E. typhi*, and *B. oedematiens*. In each case, low concentrations of 1 to 10 or 15 mg. per cent produced moderate to marked inhibition of growth. At 10 to 20 mg. per cent practically no inhibition occurred. Then from 25 to 35 mg. per cent moderate to marked inhibition again occurred, except with *B. coli*. Thus it appears that certain optimum concentrations of Sulfathalidine exist at which this drug is most effective against some organisms, rather than the degree of activity increasing with the concentration of the drug.

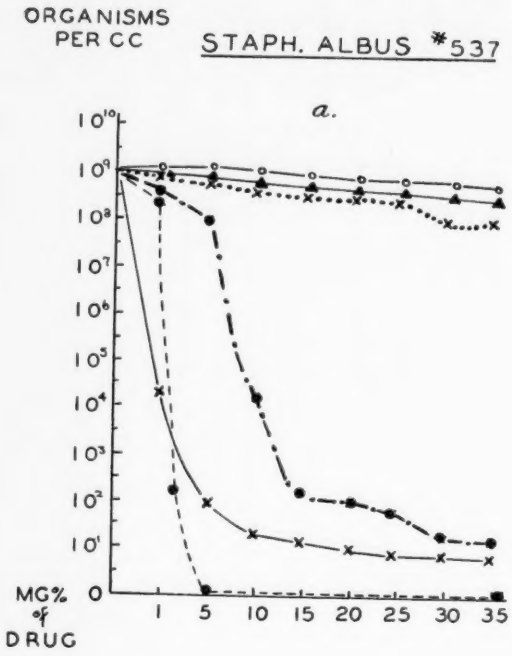
In comparing suspensions and solutions of Nisulfazole and sulfaguanidine, it was found that, although a considerable degree of inhibition of growth occurred in the suspensions, the solutions of the drugs were moderately more effective.

DISCUSSION

It is evident that Nisulfazole is a powerful bacteriostatic agent against many organisms which commonly cause, or are associated with, intestinal diseases. Its action against most of the organisms tested is superior to that of Nisulfadine, sulfapyridine, sulfaguanidine, Sulfasuxidine, and Sulfathalidine in vitro. The results indicate that Nisulfazole might be effective in the treatment of ulcerative colitis, bacillary dysenteries, paratyphoid infections, anaerobic infections, and some salmonellal food poisonings. Nisulfazole has been used in cases of ulcerative colitis with much better results than those obtained with other types of therapy at the University of Kansas Medical Center.[5] Further in vivo studies and clinical investigations are needed to establish the true value of this compound.

SUMMARY

The antibacterial activity of Nisulfazole has been compared in vitro with that of sulfathiazole, sulfapyridine, Nisulfadine, sulfaguanidine, Sulfasuxidine, and Sulfathalidine. A variety of organisms were studied, including normal inhabitants of the intestinal tract and pathogenic organisms which commonly produce diseases of the intestinal tract.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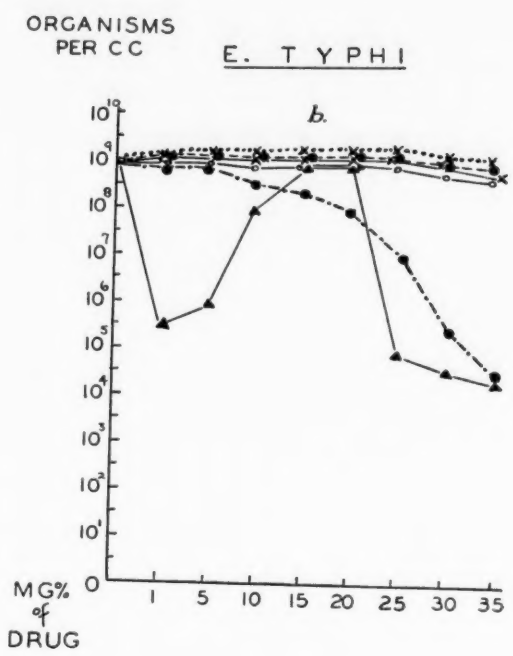
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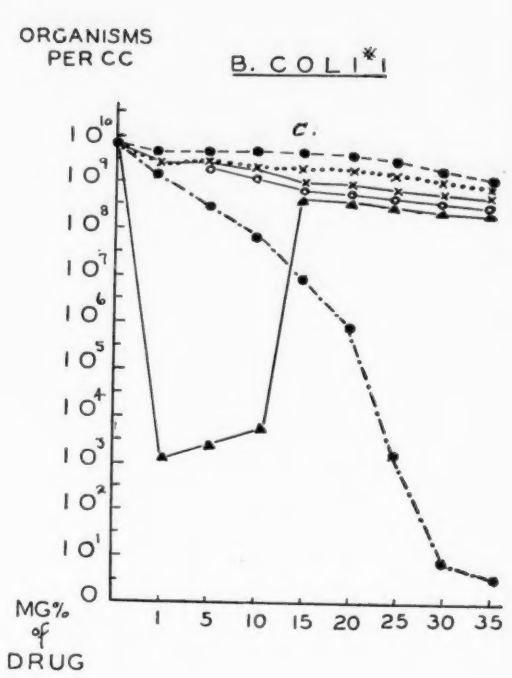
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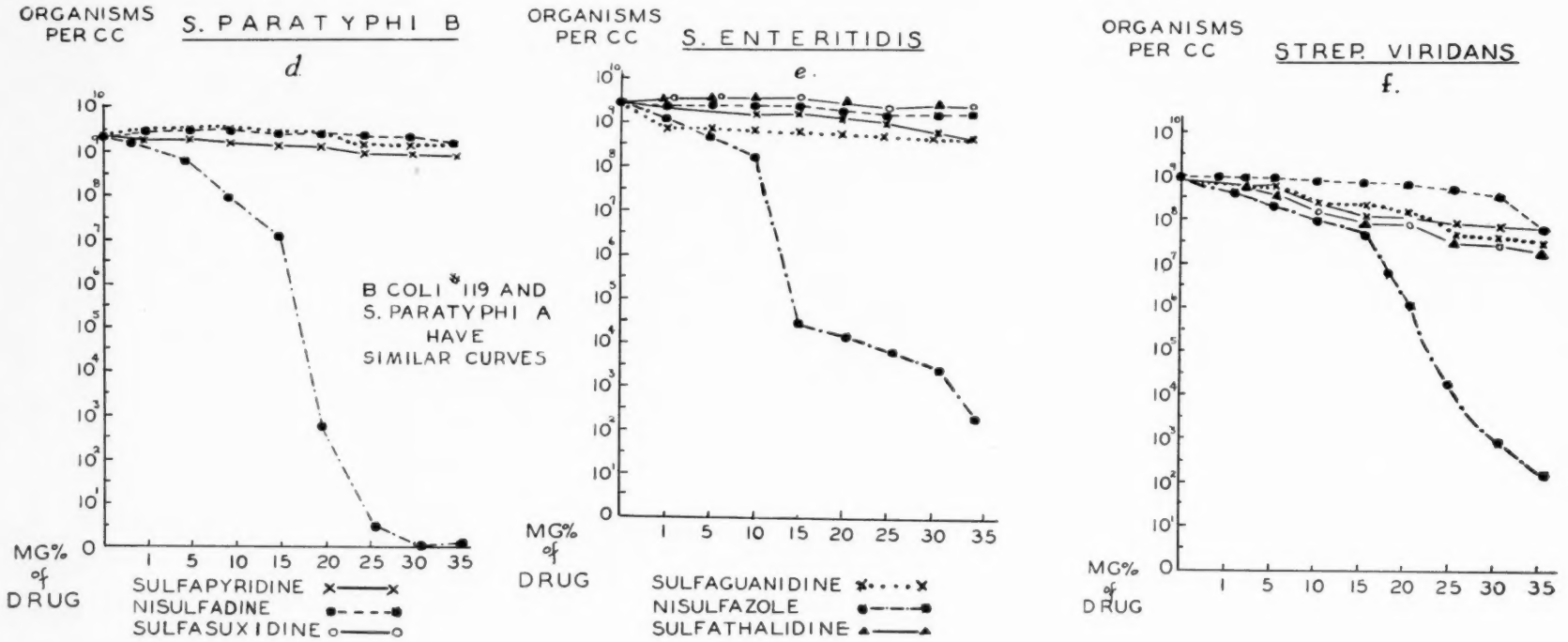
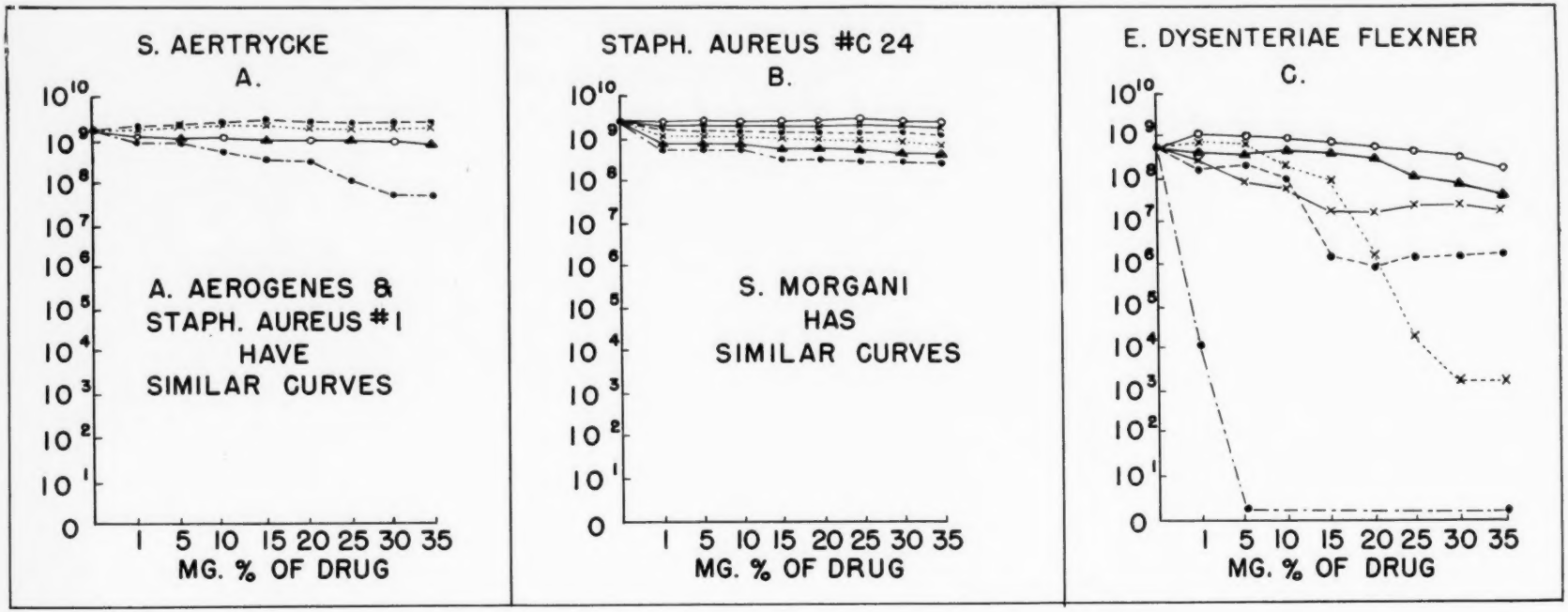


Fig. 3.—Graphs showing the comparative inhibitory actions of Nisulfazole, Sulfathalidine, Sulfasuxidine, Nisulfadine, sulfapyridine, and sulfaguanidine on the growth of: *a, Staph. albus* 537; *b, E. typhi*; *c, B. coli* 1; *d, S. paratyphi* A, *S. paratyphi* B, and *B. coli* 119 (curves for each organism similar; Sulfasuxidine and Sulfathalidine not tested); *e, S. enteritidis*; *f, Str. viridans.*


S. AERTRYCKE
A.
A. AEROGENES &
STAPH. AUREUS #1
HAVE
SIMILAR CURVES
MG. % OF DRUG
STAPH. AUREUS #C 24
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E. DYSENTERIAE FLEXNER
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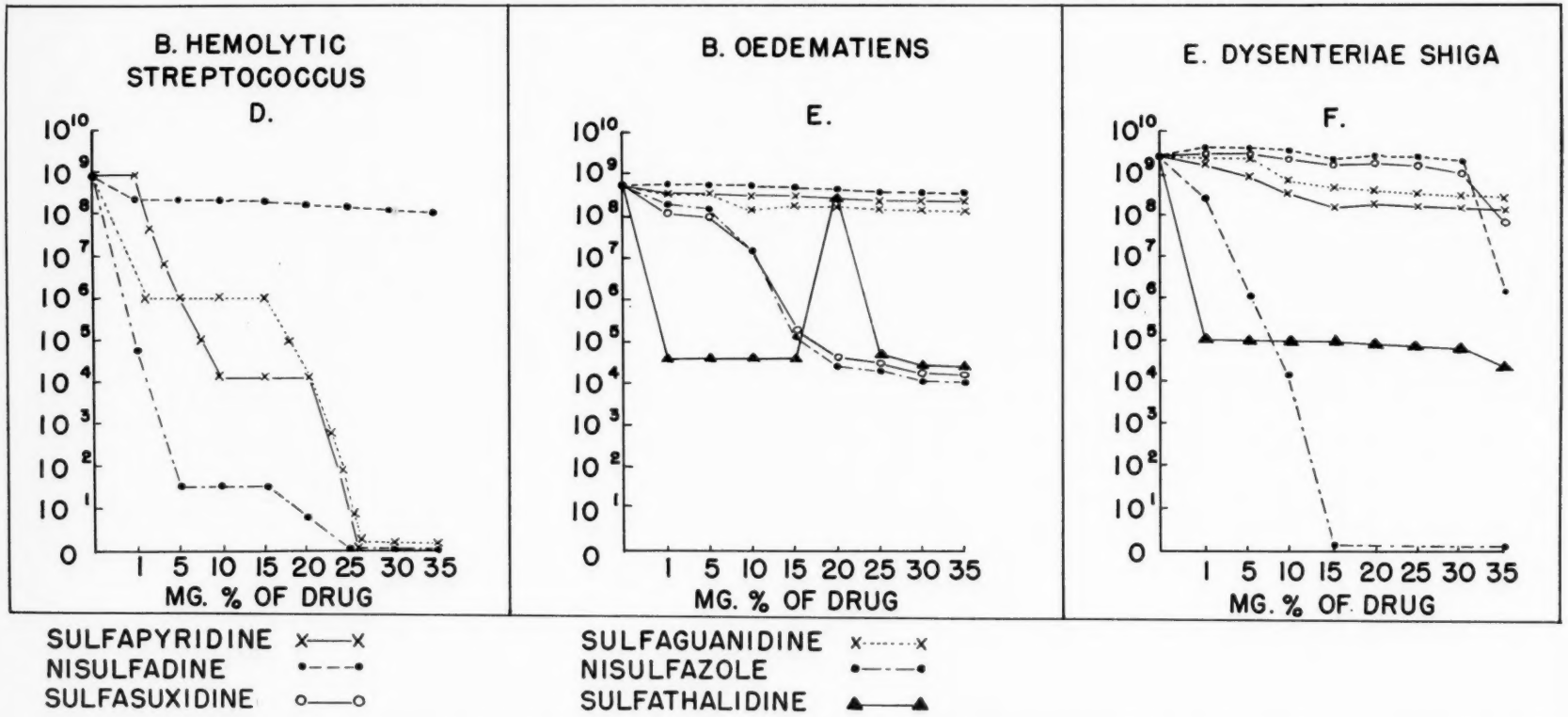

Fig. 4.—Graphs showing the comparative inhibitory actions of Nisulfazole, Sulfathalidine, Sulfasuxidine, Nisulfadine, sulfapyridine, and sulfaguanidine on the growth of: *A*, *S. aertrycke*, *A. aerogenes*, and *Staph. aureus* 1 (curves for each organism similar); *B*, *Staph. aureus* C24 and *S. morgani* (curves for each organism similar); *C*, *E. dysenteriae* Flexner; *D*, β hemolytic streptococcus, *Br. suis*, and *Br. melitensis* (curves for each organism similar; Sulfasuxidine and Sulfathalidine not tested); *E*, *B. oedematiens*; *F*, *E. dysenteriae* Shiga.

In vitro tests indicate that Nisulfazole might be more effective than the other sulfonamides tested in the treatment of various types of intestinal infections, especially ulcerative colitis, bacillary dysenteries, paratyphoid and anaerobic infections, and some salmonellal food poisonings.

The antibacterial action of Nisulfazole is not entirely dependent upon its reduction product, sulfathiazole, but is due to the combined action of sulfathiazole with the nitro compound itself, or with some intermediate reduction product.

Suspensions of Nisulfazole and sulfaguanidine are less effective in inhibiting growth of organisms than are solutions of these drugs.

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STUDIES ON LYMPHOCYTES FROM PERSONS TREATED WITH RADIOACTIVE IODINE

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WHEN phase microscopy or supravital staining is used, 34 per cent of normal lymphocytes of human beings are found to contain 1 to 8 refractile cytoplasmic granules; these were best described by Gall[1] in 1936. In 1947 Dickie and Hempelmann[2] reported striking increases in the number and frequency of these granules in the lymphocytes of persons who had been exposed to ionized radiation. Despite the potential importance of this observation, confirmatory or contradictory reports have not been seen.

METHODS AND MATERIALS

Samples of blood were obtained from capillaries of two patients having cancer of the thyroid and from eight patients having exophthalmic goiter before the administration of therapeutic doses of radioactive iodine (I^{131}) and at varying intervals thereafter (Table I). Further control studies were made on blood from normal persons and from patients with various other diseases (Table II). Samples also were taken from three technicians who had been exposed to irradiation from eight to twenty-seven years (Table III) and from four patients who were receiving massive doses of roentgen rays in treatment for malignant diseases (Table IV).

In each sample the number of refractile granules in 50 or more lymphocytes was counted. All counts were made on preparations of unstained blood unless otherwise noted. These preparations were made by touching a minute drop of blood to a thoroughly cleaned and dried No. 0 cover slip. The cover slip was inverted on a clean slide and was promptly rimmed with petroleum jelly. The preparation was examined at room temperature soon after the sample was obtained. Notations were made regarding the condition of the cell, that is, whether or not vacuolization, fine granulations, motility, degeneration of the mitochondria, or any other unusual phenomenon was present.

In addition to the living unstained preparations, supravitally stained preparations were made on approximately half of the samples. These preparations were used for comparison with the unstained and for study of the types of cytoplasmic inclusions. The supravital preparations were made by standard techniques, as described in detail by Cunningham and Tompkins,[3] except that the cleansing agent, Paragon,‡ was used on the slides.

For supravital staining, stock solutions of saturated stains were kept on hand. From these a dilute mixture was prepared; slides were stained with this latter solution and stored until needed. The stock solution of neutral red was prepared by adding 125 mg. of neutral red§ to 50 ml. of absolute alcohol in a brown, rubber-stoppered vaccine bottle. The stock solution of Janus green B was prepared by adding 125 mg. of Janus green B‖ to 62.5 ml. of neutral absolute alcohol. The solution prepared for use contained 1 ml. of neutral red and 0.1 ml. of the stock solution of Janus green B to 10 ml. of neutral absolute alcohol.

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TABLE I. PERCENTAGES* OF LYMPHOCYTES CONTAINING REFRACTILE GRANULES: BEFORE AND AFTER TREATMENT WITH I^{131}

CASE	DAY OF TREATMENT	NUMBER OF LYMPHOCYTES†	LYMPHOCYTES (%)			DIAGNOSIS AND DOSAGE OF I^{131}
			WITHOUT GRANULES	1 OR 2 GRANULES	MORE THAN 2 GRANULES	
1	Before treatment	1,000	43	55	2	Carcinoma of thyroid
	3	1,200	50	48	2	50 mc.
	6	800	60	38	2	
	8	700	56	34	10	
	10	400	74	26	0	
2	Before treatment	1,400	60	40	0	Exophthalmic goiter
	2	1,600	62	36	3	20 mc.
	4	1,200	54	44	2	
	8	900	42	54	4	
	10	1,000	42	53	6	
3	Before treatment	2,500	56	41	2	Exophthalmic goiter
	3	1,900	54	44	4	5 mc.
	5	2,100	58	42	0	
	8	1,500	42	56	4	
	15	1,700	50	50	10	
	29	2,000	58	42	0	
4	Before treatment	1,900	56	41	3	Adenomatous goiter
	3	3,400	68	26	6	25 mc.
	8	3,200	38	55	7	
	10	4,100	50	45	7	
	14	2,400	47	49	5	
5	Before treatment	2,800	69	27	3	Exophthalmic goiter
	6	2,200	52	40	8	3.5 mc.
6	Before treatment	2,000	42	48	10	Exophthalmic goiter
	6	2,000	39	52	8	18 mc.
7	Before treatment	2,100	64	34	2	Exophthalmic goiter
	6	2,000	60	40	0	8 mc.
8	Before treatment	1,800	38	50	12‡	Exophthalmic goiter
	7	1,700	50	32	18‡	8 mc.
9	Before treatment	1,100	54	42	4	Exophthalmic goiter
	12	1,300	42	50	8	6.5 mc.
	45	1,000	62	38	0	
10	Before treatment	600	66	32	2	Carcinoma of thyroid
	6 hours	1,100	54	42	4	25 mc.
11	Before treatment	--	--	--	--	Carcinoma of thyroid
	9	900	78	22	0	140 mc.

*All percentages based on a count of at least 50 lymphocytes.

†Number of lymphocytes in all tables refers to absolute count, meaning number of leucocytes times per cent of lymphocytes in Romanowsky smear.

‡These counts were on supravitally stained preparations only; such counts tend to be higher because of the confusing dye inclusions.

Differential leucocyte counts were made of 100 cells in the nonstained living or in the supravitally stained preparation and of 200 cells in a standard Romanowsky stained film for orientation and comparison. Routine counting of leucocytes was performed by technicians. The absolute count was calculated by multiplying the number of leucocytes by the percentage of lymphocytes in the Romanowsky stained film.

All preparations were studied with the Spencer phase microscope using phase optical diffraction plates $1B - 0.25\ \lambda$, $0.14A - 0.25\ \lambda$, or $0.2A + 0.25\ \lambda$. The objective magnification was 97 ×, oil immersion, and the oculars were 15 ×. The $1B - 0.25\ \lambda$ (low contrast) plate was used routinely for counting inclusions, while the $0.14A - 0.25\ \lambda$ (dark contrast) plate was used chiefly for further study of cellular structure and for photography.

TABLE II. PERCENTAGES OF LYMPHOCYTES CONTAINING REFRACTILE GRANULES: NORMAL CONTROLS AND PATIENTS HAVING VARIOUS PATHOLOGIC CONDITIONS

CASE	DIAGNOSIS AND OTHER CONDITIONS	NUMBER OF LYMPHOCYTES	LYMPHOCYTES (%*)		
			WITHOUT GRANULES	1 OR 2 GRANULES	MORE THAN 2 GRANULES
12	Normal	2,800	26	58	16†
	Normal	2,000	35	62	4
	Coryza	2,100	40	46	14
	Normal	1,600	58	36	6
	Normal	3,700	66	34	0†
	4 hr. after ACTH‡	1,500	66	30	4†
13	Normal	2,500	40	58	2
	4 hr. after ACTH‡	2,100	40	46	14
14	Normal	4,500	46	50	4†
	4 hr. after ACTH‡	2,900	42	58	0†
15	Cushing's syndrome	1,300	56	29	15
16	Lymphatic leucemia	940,000	88	12	0
17	Toxic polys, grade 4	1,100	70	30	0
18	Infectious mononucleosis	4,300	80	18	2
19	Hyperlipemia§	2,000	56	36	8
20	Hyperlipemia‖	1,900	42	50	8

*All percentages based on a count of at least 50 lymphocytes.
†Supravital slide only.
‡Administration of 25 mg. of adrenocorticotropic hormone.
§Cholesterol, 860; total fats, 2,234.
‖Cholesterol, 936; total fats, 3,928.

TABLE III. PERCENTAGES OF LYMPHOCYTES CONTAINING REFRACTILE GRANULES: TECHNICIANS WHO HAD PROLONGED SLIGHT EXPOSURE TO ROENTGEN RAYS

CASE	DURATION OF EXPOSURE (YR.)	NUMBER OF LYMPHOCYTES	LYMPHOCYTES (%)		
			WITHOUT GRANULES	1 OR 2 GRANULES	MORE THAN 2 GRANULES
21	27	2,700	62	30	8
22	20	1,500	58	38	4
23	8	1,700	64	34	2

TABLE IV. PERCENTAGES OF LYMPHOCYTES CONTAINING REFRACTILE GRANULES: PATIENTS RECEIVING LARGE THERAPEUTIC DOSES OF ROENTGEN RAYS*

CASE	TREATMENT	DAY OF TREATMENT	NUMBER OF LYMPHOCYTES	LYMPHOCYTES (%)			DIAGNOSIS
				WITHOUT GRANULES	1 OR 2 GRANULES	MORE THAN 2 GRANULES	
24	11 fields, 540 r each	Before treatment	1,800	64	32	4	Seminoma
		12	700	70	25	5†	
25	4 fields, 750 r each	Before treatment	3,800	64	34	2	Carcinoma of bladder
		9	1,300	74	26	0	
26	4 fields, 500 r each	Before treatment	2,000	72	28	0‡	Carcinoma of cervix
		4	1,400	68	22	10	
27	4 fields, 1,000 r each	Before treatment	900	--	--	--§	Carcinoma of cervix
		21	300	66	28	6	

*Thrombocytopenia presumably due to excessive radiation exposure.
†Percentages in this line based on 40 lymphocytes; all others based on 50 or more lymphocytes.
‡Previous roentgen ray or radium therapy not included in this report.
§Note the lymphopenia following massive irradiation.

Photomicrographs were taken with a Bausch and Lomb Type K camera. Light was obtained from a focusing lamp with a 200 watt tungsten filament. A No. 56 G. Wratten gelatin filter was used frequently. The film was Super Panchro-Press, Type B, Eastman Kodak Company. Exposures varied from one to three seconds.

RESULTS

The refractile granules were usually distinct and identifiable. Two groups of inclusions sometimes appeared similar, the mitochondria and the degenerative vacuoles. In the fresh preparation, the mitochondria were usually fine, thread-like or rodlike forms, but as the preparation aged they tended to clump and become more granular and opaque. Frequently, they appeared to coagulate into multiple opaque granules, called the "acetic acid form" by Wiseman[4] because of the similarity to preparations fixed in acetic acid. Considerable practice was needed to separate these degenerative forms from the true, refractile granules.

The degenerative vacuoles were seen particularly in heavily stained preparations, where the dye often accumulated in the Hof of the nucleus forming the "segregation apparatus" of the early users of supravital staining. In unstained preparations these areas were less dense and more readily separated from the opaque refractile granules. In contrast to the vacuoles and mitochondria, the refractile granules were constant, refractile, and opaque. When observed for as long as ninety-six hours, they did not change in appearance or number, although the bulk of the preparation had degenerated to a mass of granular and vacuolar debris.

Significant change in the number of refractile granules did not occur in the lymphocytes of any of our patients in the irradiated groups. Instead, there was remarkable constancy in the number of granules in each cell and in the number of cells containing granules from day to day and under varying conditions. This was equally true of patients who received therapeutic doses of radioactive iodine, of patients who had received large doses of roentgen rays in short courses, and of technicians who had suffered chronic irradiation. It was noted that the degree and speed of degeneration of the cytoplasm varied greatly with the thickness of the preparation and that they were acutely accelerated by any slight trauma, such as accidental touching of the cover slip. Likewise, variations in temperature affected the rate of degeneration; sometimes a cell which was kept in the beam of the microscope lamp for a long time seemed to fry. Most important, however, was the age of the preparation. The character of the cytoplasm changed rapidly as the cells sickened in the nonsustaining environment of glass. Because of these many variables the rate of degeneration did not seem measurable. In summary, the effects of trauma in vitro far overshadowed the relatively slight effects of irradiation in vivo. Hence, attempts to correlate the various degenerative forms of lymphocytes with the degree of exposure to radiation failed.

COMMENT

Because of the many variables of intensity in radiation, the duration of exposure and the depth of penetration, comparison between this series and that of Dickie and Hempelmann[2] was difficult. It was apparent, however, that most

patients concerning whom data are reported herein received much greater exposure than the estimated 3 r per month of the most heavily exposed group reported by Dickie and Hempelmann. On the basis of an estimated equivalent exposure of 1 r per millicurie of radioactive iodine, the patients in our series received a total of 3 to 50 r to the body. The study of the patients who received massive therapeutic doses of roentgen rays and the technicians who had suffered chronic exposure for many years was included to bring out the factors of intensity and duration of exposure. In neither group were changes in the refractile granules found.

This study differed from that of Dickie and Hempelmann in that they used "much stronger solutions of stain than those recommended in the literature." They presumed that the scattered light at their altitude modified the dye in some unknown manner. On the other hand, in our study relatively dilute solutions were used, because excessive staining loaded the cells with nonspecific inclusions and aggregations of dye. The true refractile granules are readily visible and less likely to be mistaken in the fresh, unstained state under the phase microscope.

Our observations are in accord with Gall's statement that the refractile granules are remarkably constant; the increments after irradiation reported by Dickie and Hempelmann were not observed.

SUMMARY

A study was made to determine whether or not the number and frequency of refractile cytoplasmic granules known to be present in 34 per cent of normal lymphocytes increase on exposure of the host to ionized radiation. A significant change in the number or frequency of the refractile granules in lymphocytes from persons exposed to ionized irradiation was not found. A search was made for other concise or specific qualitative cytologic effects of irradiation, but none was found. The refractile granules were best studied in fresh, unstained preparations by phase microscopy.

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GELFOAM AND THROMBIN IN GASTRODUODENAL BLEEDING

An Experimental Study

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IN THE past five years much interest has been shown by surgeons in the use of thrombin and Gelfoam as hemostatic agents. As a result of experimental and clinical studies with Gelfoam by many workers,[1-6] it was conclusively shown that this substance was nonantigenic and easily absorbed by the body. Its hemostatic action when applied to bleeding tissue was found to resemble the effect of human fibrin both clinically as well as histologically. The use of Gelfoam in neurosurgery, genitourinary surgery, and abdominal surgery was found to be superior to human fibrin used for the same purpose because it was easily obtained, relatively inexpensive, and very easily handled. When used as a hemostatic agent in bleeding areas, the Gelfoam was directly applied and pressure was maintained because it was believed that it was necessary for blood to fill the spaces of the gelatin sponge (Gelfoam) before hemostasis could occur.

The recent studies by Daly[7] in the treatment of gastroduodenal bleeding using buffer thrombin solution showed promise. Although all patients with gastroduodenal hemorrhage did not stop bleeding by the use of the buffer thrombin, it was believed that the thrombin was a useful addition to our armamentarium in the control of such bleeding.

The remarkable hemostatic action of the Gelfoam and thrombin used either alone or in combination by previous workers in various fields of surgery impelled the idea of using the Gelfoam and thrombin as a hemostatic agent in the control of gastroduodenal bleeding. Since there had been ample demonstration that the behavior of the Gelfoam within tissue was the same as that of human fibrin, the obvious conclusion was reached that it might not be necessary to apply Gelfoam sponge to the bleeding site with pressure to obtain hemostasis since a powder could be made of the Gelfoam. This powder should behave like pure human fibrin. In order to determine the effectiveness of this hemostatic agent as well as its fate in the stomach, this experimental study was carried out. It seemed only reasonable that if thrombin were of value in the control of gastroduodenal bleeding, surely the addition of Gelfoam, which behaves like human fibrin, should be much more effective since we would have two substances that have been shown to participate in clot formation—a fibrin-like matrix (Gelfoam) and the thrombin.

METHOD

Six dogs were used in this experimental study. Each dog was anesthetized by intravenous sodium Nembutal using 0.065 Gm. per 2 kilograms of body weight. The Gelfoam was specially prepared* in the form of a very light dry powder. The thrombin was supplied*

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in vials of 1,000 Iowa units. The contents of a vial of thrombin were dissolved in 200 c.c. of water thus giving a dilution of 5 Iowa units of thrombin per cubic centimeter of water. Fifty cubic centimeters of this solution containing 250 Iowa units of thrombin were then used with each 2 tablespoonsful of the Gelfoam powder.

EXPERIMENTAL

Experiment 1.—To determine the behavior of the Gelfoam powder alone in the stomach and to determine the effect of (a) thrombin alone upon venous bleeding, (b) thrombin alone on small arterial bleeding, (c) Gelfoam alone on venous bleeding, (d) Gelfoam alone on small arterial bleeding, (e) Gelfoam and thrombin upon venous bleeding, and (f) Gelfoam and thrombin upon small arterial bleeding.

Before anesthetizing the dog, 4 tablespoonsful of the Gelfoam powder were fed to the animal in an effort to determine what the Gelfoam would do in its dry state. The abdomen was then opened and a 2 in. incision made in the anterior stomach wall. A section of gastric mucosa 2.5 cm. in diameter was then excised from the posterior wall of the stomach. Free bleeding began. Thrombin solution (5 Iowa units per cubic centimeter) was applied to the bleeding area. The time required for the bleeding to cease was noted. The clot was then rubbed off and free bleeding was re-established. Dry Gelfoam powder was then applied to the denuded area and the speed of clot formation noted. This clot was then removed and a mixture of Gelfoam and thrombin of the same concentration as in the previous test was applied to the bleeding area and the speed of clot formation noted.

This procedure was repeated three times by denuding the same sized section of mucosa in different portions of the stomach.

A deeper section of tissue of the same size was then excised from the antral portion of the stomach and arterial bleeding was produced. The same procedures were then carried out as in the experiments with venous bleeding.

Results: From this experiment, it appeared that Gelfoam and thrombin in combination were much more effective than either the thrombin or Gelfoam alone in the ability to stop either venous or small arterial bleeding. The type of clot formed by Gelfoam or Gelfoam and thrombin was much more desirable than that formed by thrombin alone. Gelfoam and thrombin invariably caused the formation of a flat, tenacious clot that could be removed with some effort. This type of clot would be quite difficult to dislodge. The clot formed by the thrombin alone was of a mushroom type and loosely attached. Because of this, it could easily be dislodged. When swallowed as a dry powder, the Gelfoam was found to spread evenly over the gastric mucosa in a finely granular form.

Experiment 2.—To determine the best method of administering the Gelfoam, Gelfoam and thrombin, and the thrombin. The speed of clotting and the type of clot formed was noted.

A balloon-tipped, mercury-weighted tube was passed into the stomach of an anesthetized animal. An attempt to blow Gelfoam powder through the tube into the stomach failed because the slightest moisture caused the powder to become sticky. This resulted in plugging of the tube. Gelfoam powder mixed with water was placed in a syringe for injection through the tube. The Gelfoam tended to separate out and could be injected only with difficulty. Gelfoam powder mixed with mineral oil and shaken formed a suspension which could be injected easily through the tube. A sigmoidoscope was inserted through a gastrostomy opening in the anterior stomach wall after a section of mucosa 2.5 cm. in diameter was excised from the posterior wall and free venous bleeding produced. Thrombin solution (250 Iowa units) was injected through the tube and its effect noted upon the bleeding. The same observations were made after the injection of Gelfoam in water and Gelfoam in oil, and finally Gelfoam and thrombin in water and in oil.

Results: It was evident from this experiment that to be effective the hemostatic agent must come into direct contact with the source of the bleeding. The mercury-weighted tube was found to lie in the fundic pouch of the stomach with the dog flat on its back. As a result of this, all material injected through the tube would pool in the left paravertebral gutter within the fundic pouch. Since the bleeding area was made on the posterior antral wall, none of the hemostatic material could possibly exert its effect. When the tube was threaded through the pylorus and the same experiment repeated, the most rapid clot formation resulted when Gelfoam and thrombin were used together. The type of clot formed was the most desirable because it was flat, firm, and very tenaciously attached to the underlying tissue. The thrombin alone resulted in the formation of a friable, mushroom type of clot that could easily be brushed off.

Experiment 3.—To determine whether the observations noted in our previous studies relative to the speed of clot formation and the type of clot formed could be substantiated. Experiments 1 and 2 were performed again in exactly the same fashion.

Results: Essentially the same results as noted in Experiments 1 and 2 were obtained with regard to both the speed of clot formation and the type of clot formed.

Experiments 4, 5, and 6.—To recheck the previous observations and test the use of milk and cream as a vehicle for the Gelfoam.

Results: The results of these three experiments again substantiated the previously obtained results. Milk and cream furnished an excellent vehicle for Gelfoam powder. Clot formation was most rapid when Gelfoam powder and thrombin were used together and the type of clot was the most satisfactory.

DISCUSSION

The great interest in the new hemostatic agents and the excellent results obtained with their use both experimentally and clinically have furnished an impetus for the study of the effect of these agents either alone or in combination in those areas of the body in which ligation of bleeding vessels is not feasible. The promising results reported with the use of buffer thrombin solution in the treatment of bleeding gastroduodenal lesions has raised the question as to whether Gelfoam and thrombin together might not be better for this purpose since two substances that are known to accelerate clot formation are thus used. The Gelfoam behaves like human fibrin in the body with the result that it furnishes a matrix for the thrombin. It would seem reasonable that if thrombin were effective, Gelfoam and thrombin should be much more effective.

Clotting in dogs is very rapid. Preshaw[8] demonstrated in twenty dogs that it was difficult to exsanguinate the animal despite the amount of mucosa excised. Our experience substantiated this. For this reason, it is difficult to evaluate the speed of clotting by excision of gastric or duodenal mucosa when testing various hemostatic agents. The only rational criterion would be to determine the relative speed with which clot formation occurred as noted by different groups of observers. This we attempted to do.

Four observers agreed that the use of Gelfoam and thrombin in combination was far superior as a hemostatic agent to either Gelfoam or thrombin alone.

It is essential that the hemostatic agent bathe the bleeding area if a hemostatic effect is to be obtained. The implications of this observation are that it is of little use to give any of these hemostatic agents orally in human beings when the lesion cannot be bathed by the material because of its position. In such cases, positioning the patient is of value when the site of the bleeding is known.

CONCLUSIONS

Thrombin solution when directly applied to an oozing, denuded lesion in the stomach of dogs resulted in the formation of a mushroom type of clot loosely attached. The oozing is effectively checked in a shorter period of time than it would be in control animals in which nothing had been applied.

Gelfoam powder alone when applied to the denuded, oozing area in the stomach resulted in much more rapid clot formation and more rapid cessation of the bleeding than thrombin. The clot was flat and firm.

Gelfoam and thrombin combined was by far the best hemostatic material for the control of bleeding, denuded, gastric lesions. Not only was the bleeding most rapidly checked, but also the clot formed was flat, firmly attached, and very tenacious so that dislodgement was difficult.

Oil or milk and cream constitute the best vehicle for carrying the Gelfoam and thrombin.

Although the results in dogs appear very promising, the use of Gelfoam and thrombin in human beings for the control of gastroduodenal bleeding involves other problems such as the great difference in coagulability between human and dog blood, the position of the ulcer, the fact that in older human beings such bleeding lesions are often indurated ulcers on the posterior wall and, finally, the fact that a sclerotic branch of the gastroduodenal or pancreaticoduodenal artery is eroded.

The results obtained in dogs warrants the use of Gelfoam and thrombin in the treatment of bleeding gastroduodenal lesions in human beings and a further study along clinical lines.

Dr. L. Gravelle and Dr. D. E. Preshaw assisted in some of the surgical procedures and acted as observers. The facilities of the Wayne University laboratory were loaned through the courtesy of Professor C. G. Johnston. The material used was specially made up in this new powdered form and supplied by the Upjohn Company.

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THE LONG-TERM EVALUATION OF FOLIC ACID IN THE TREATMENT OF PERNICIOUS ANEMIA

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THE therapeutic history of pernicious anemia may be divided into three parts. The first of these is the pre-liver and the second the liver era, while the third encompasses the period devoted to the search for a substance more finite than liver.

In 1945, folic acid (pteroylglutamic acid) was introduced[1] as a possibly specific substance for the treatment of pernicious anemia. An avalanche of reports[2] soon followed. These were based on small numbers of cases which were studied for short periods of time and presented a unanimity of opinion that folic acid was an effective therapeutic agent. It was not until some time later that warnings began to appear in the literature calling attention to the deleterious and sometimes explosively harmful effects of folic acid on the neurologic manifestations of the disease.[3]

METHODS

Because the natural history of pernicious anemia is characterized by spontaneous exacerbations and remissions, we felt that long-term studies were necessary to critically evaluate any new therapeutic agent. Accordingly, early in 1946, ninety-eight patients with pernicious anemia were selected from the outpatient Anemia Clinic of the Cook County Hospital for the evaluation of the therapeutic efficacy of folic acid. Their clinical course, liver extract requirements, neurologic and hematologic status, and other characteristics were well established, the patients having been followed by the same group of observers, and maintained in remission with liver extract, for periods ranging from months to years. All were in complete hematologic and neurologic remission. The folic acid was administered in a daily dose of 5 mg. orally. The patients were seen at four-week intervals for interviews and neurologic and hematologic examinations. The criteria for neurologic relapse were the objective findings of posterior and/or lateral column dysfunction, namely hyperactive deep reflexes, positive Romberg sign, and impairment of vibratory sensation. The development of macrocytosis and a progressively falling erythrocyte level with a rising color index were considered evidences of hematologic relapse. The highest erythrocyte level at which folic acid therapy was discontinued in this series was 3.7 million. The marrow was examined in five patients during relapse and in each instance was found to be characterized by the presence of pernicious anemia type of erythro- and granulopoiesis.

The present report summarizes the observations made during the three and a half years that this study has been in progress. Of the ninety-eight patients originally selected, fifty-eight relapsed, twenty-six interrupted therapy, and only twelve have been maintained in satisfactory remission for three and a half years (Fig. 1).

From the Hematology Laboratory and the Hektoen Institute for Medical Research of the Cook County Hospital.

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*Formerly Fellow of the Hematology Research Foundation.

RESULTS

Relapses (Fifty-eight Cases).—

(a) Neurologic: There were twenty-three in this group. Four relapsed in less than one year, and nineteen between one and two years. The abnormal neurologic findings were not reversible in eight cases in spite of intensive liver therapy for six months or more.

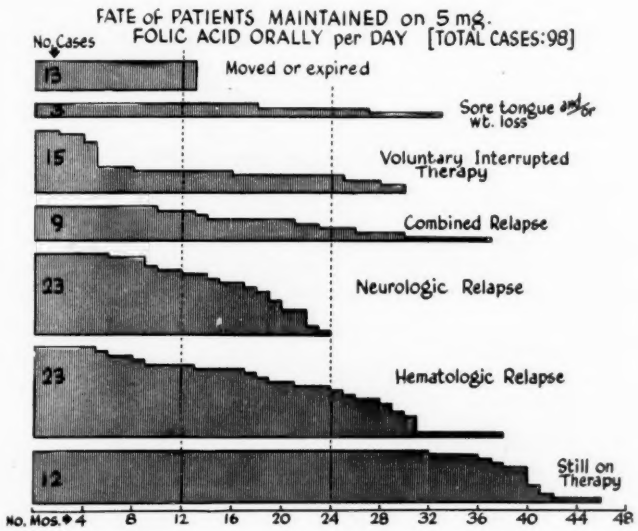

Fig. 1.—Relapse patterns observed in patients with pernicious anemia treated with folic acid.

(b) Hematologic: There were twenty-three cases in this group, too. Four of these relapses occurred during the first twelve months, eight during the second twelve months, and eleven after more than two years of folic acid administration. This type of relapse was completely reversed by parenteral liver.

(c) Combined Hematologic and Neurologic Relapse: Nine cases. For the most part these relapses were primarily hematologic and showed a pattern similar to that seen for the hematologic type alone. One patient relapsed during the first twelve months, three during the second twelve months, and five after periods longer than two years. All of these relapses were reversible.

(d) Sore Tongue and/or Weight Loss: Three cases. These relapses occurred at eighteen, twenty-six, and thirty-three months and were successfully combatted with intramuscular liver.

Interrupted Therapy (Twenty-six Cases).—

(a) Fourteen patients voluntarily interrupted their treatment, many after insisting that they did not feel as well while taking the "pills" as they had with the "shots." In the absence of positive objective findings they were not changed back to liver and so disappeared.

(b) In twelve patients treatment was discontinued because of death, moving from the city, or other unrelated cause.

Satisfactory Maintenance (Twelve Cases).—

Twelve patients have remained well on uninterrupted folic acid therapy during the three and a half year study period.

COMMENT

The optimism attending the early studies with folic acid in pernicious anemia was sharply curtailed in 1947 by an editorial, "A Warning Regarding the Use of Folic Acid."[4] This editorial stressed the untoward effects of folic acid on the central nervous system in pernicious anemia. At the time, we were well into our long-term project and had already collected sufficient evidence to

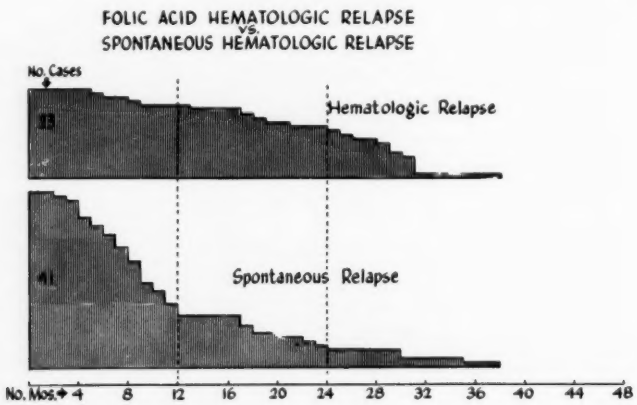

Fig. 2.—Comparison of folic acid and spontaneous hematologic relapse in pernicious anemia.

condemn folic acid on hematologic as well as neurologic grounds. The effect on these two systems, however, differs as can be seen by comparing the hematologic and neurologic relapse patterns. (Fig. 1.) Eighty-three per cent of the neurologic relapses occurred between twelve and twenty-four months and none after two years, while almost as many hematologic relapses occurred after two years of treatment as were seen at shorter intervals. This temporal relation, the sometimes precipitous onset, and the frequent irreversibility of the neurologic component of the disease suggests a mechanism other than that operating in the hematologic realm. Whether this difference depends on the interference of the folic acid with the utilization of glutamic acid by nerve tissue, as suggested by Ross and associates,[5] an unbalance in vitamin utilization,[6] or some other cause, remains an unsettled problem.

The effectiveness of folic acid in maintaining hematologic remission is shown in Fig. 2. Here, spontaneous relapses are compared with those occurring during folic acid therapy. The former group represents the natural history of the patient who discontinues therapy after a liver-induced remission.[7] The two types differ remarkably. Without any treatment most patients relapse in less than one year, while those who take folic acid relapse, for the most part, after the first year. This is one of the reasons the early reports on the therapeutic efficacy of folic acid were premature. We believe that folic acid does at first

produce a remission (though incomplete) either by utilizing or making utilizable other growth substances still available; but with their exhaustion its protective effect seems progressively to diminish. In short-range experiments encompassing weeks or months, folic acid is a fairly satisfactory therapeutic agent, but over a longer span of time its therapeutic inadequacy becomes apparent.

With the enthusiasm attendant upon the discovery of a new agent for the treatment of pernicious anemia, the fact is lost sight of that there is a tremendous variability inherent in the disease as relates to remissions and relapses. The realization that any new therapeutic agent introduced for the treatment of pernicious anemia must be evaluated in many patients over a long period of time before the full effectiveness and safety from harmful side reactions can be accepted must permeate our thinking, in order that extravagant and premature claims may be prevented from gaining circulation and credence.

SUMMARY AND CONCLUSIONS

1. Ninety-eight patients with pernicious anemia were studied during a three and a half year period in order to evaluate the therapeutic effectiveness of 5 mg. folic acid per day orally.

(a) Twenty-three patients relapsed hematologically, half in less and half in more than two years. All hematologic relapses were reversible.

(b) Twenty-three patients relapsed neurologically, all in less than two years. Not all relapses were reversible.

(c) Nine patients had combined neurologic and hematologic relapses.

(d) Twelve patients have been satisfactorily maintained on folic acid throughout the study period.

2. Folic acid, as used in this study, is an unsatisfactory therapeutic agent for the maintenance of patients with pernicious anemia.

3. In view of the natural history of pernicious anemia, any new therapeutic agent must be evaluated over a long period of time and on many patients before it can be considered safe, effective, and superior to tested, available medications.

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THE EFFECT OF ADRENOCORTICOTROPIC HORMONE UPON UROPEPSIN EXCRETION

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THE multiplicity of diseases affected by adrenocorticotropic hormone attests to its diverse influence upon the body. Peptic ulcer is considered to be an "alarm" phenomenon, and since ACTH is a method of producing "alarm"[1] (bypassing stimulation from higher thalamic centers), studies were undertaken to determine the effect of ACTH upon gastric function.

Uropepsin is a proteolytic enzyme known to be present in the urine of healthy persons in amounts quantitatively constant from day to day and unaffected by urine volume, pH, or specific gravity.[2] That it has its origin in the stomach is evidenced by the disappearance of the enzyme following total gastrectomy in animals,[3, 4] and by its absence in the urine of patients with pernicious anemia and an atrophic gastric mucosa.[5, 6, 7]

It has been suggested that pepsinogen, the precursor of the pepsin in gastric juice, is also the source of uropepsin; this seems likely, since the intravenous administration of pepsinogen increases the uropepsin output in the urine, while the oral or intravenous administration of pepsin is without effect.[7, 8] The chief cell, therefore, may be considered to have both an endocrine and an exocrine function; uropepsin seemingly is a measure of its "endocrine" activity, while gastric pepsin represents the direct secretion of the gastric glands into the stomach lumen.[8]

METHOD

Twenty-four hour urine specimens were collected and refrigerated immediately. Uropepsin activity was measured by a modification of the methods described by Bucher,[6, 9] Mirsky,[8] and Anson.[10] An aliquot of 20 c.c. of urine was adjusted to pH 1.5 and made up to 25 c.c. with distilled water as described by Mirsky.[8] One cubic centimeter of this acidified urine was incubated with 5 c.c. denatured acidified hemoglobin substrate at 37° C. for thirty minutes. Ten cubic centimeters of trichloracetic acid were added to stop the reaction, and the concentration of tyrosine-like substances in the filtrate was determined by the Folin-Ciocalteu procedure[11] with the use of a photoelectric colorimeter.

To correct for chromogenic substances present in the urine and substrate prior to proteolysis, 1 c.c. of the acidified diluted urine and 5 c.c. of hemoglobin substrate were added directly to trichloracetic acid and the color was determined as described. The

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difference in colorimeter readings between the incubated and unincubated specimens indicated the production of tyrosine-like substances resulting from proteolysis. Peptic units were then calculated with the aid of a standard curve.

The unit of activity of uropepsin was defined arbitrarily in this study as that amount which, during thirty minutes of incubation at 37° C. in the standard assay, released 0.04 mg. of tyrosine-like substance. Results were expressed as units per cubic centimeter of undiluted urine or units excreted in twenty-four hours.

Gastric juice pepsin was determined by the method of Bucher, Grossman, and Ivy.[9]

RESULTS

Normal Uropepsin Excretion.—Although the uropepsin excretion among fifty normal subjects varied from 1,050 to 3,100 units in twenty-four hours, the excretion was constant for each individual from day to day with a standard deviation which did not exceed 12 per cent, regardless of urine volume (Table I). The twenty-four hour urinary excretion of uropepsin was independent of fluctuations in urine volume; when the urine volume rose, the concentration fell, and conversely, so that the total daily output remained constant.

TABLE I. DAILY VARIATION IN THE 24-HOUR URINARY EXCRETION OF UROPEPSIN IN NORMAL SUBJECTS

PATIENT	UROPEPSIN EXCRETION (UNITS/24 HR.)	MEAN	STANDARD DEVIATION (%)	URINE VOLUME (C.C.)	PATIENT	UROPEPSIN EXCRETION (UNITS/24 HR.)	MEAN	STANDARD DEVIATION (%)	URINE VOLUME (C.C.)
	2,600			900		1,800			600
B. A.	2,000	2,283	238	720	R. I.	2,000	1,877	100	4,000
	2,250			1,800		1,760			800
						1,950			400
	2,040			1,120		2,140			1,400
G. O.	1,700	1,913	152	800	G. Z.	2,420	2,268	115	2,450
	2,000			1,000		2,246			1,000
	1,460			900		3,100			1,550
R. O.	2,000	1,748	222	750	S. B.	2,910	3,042	79.3	1,150
	1,785			1,650		3,100			2,600
						3,060			1,800
	2,500			810					
B. R.	2,700	2,700	161	960					
	2,900			1,100					

The administration of 100 mg. of ACTH daily to a normal subject produced an immediate and marked increase in the twenty-four excretion of uropepsin. (Fig. 1, Patient O. T.) The urinary excretion of uropepsin during the control period averaged 1,725 units. It rose to a level of 3,200 units within twenty-four hours of ACTH administration and increased steadily until a level of 6,401 units was attained on the seventh day. The uropepsin excretion fell to 3,504 units five days after ACTH was discontinued and returned to a normal level of 1,500 units on the sixteenth day.

The concentration of uropepsin measured in units per cubic centimeter of urine increased to 3.2 units within twenty-four hours of ACTH administration,

from the initial level of .97 units, and reached its peak of 3.7 units on the seventh day. Upon discontinuing ACTH, the concentration of uropepsin returned to its original level.

Similar marked and consistent increases in the twenty-four hour excretion of uropepsin after ACTH administration were noted in Patients 2 to 11 (Table II). The mean twenty-four hour uropepsin excretion increased from the control range of 1,600 to 3,180 units to values of 4,100 to 6,800 units. The mean concentration of uropepsin increased concurrently from initial readings of 0.66 to 3.1 units per cubic centimeter to a level of 1.4 to 6.6 units. In all instances, the mean twenty-four hour uropepsin excretion and the concentration of uropepsin returned to the control level after the ACTH administration was discontinued.

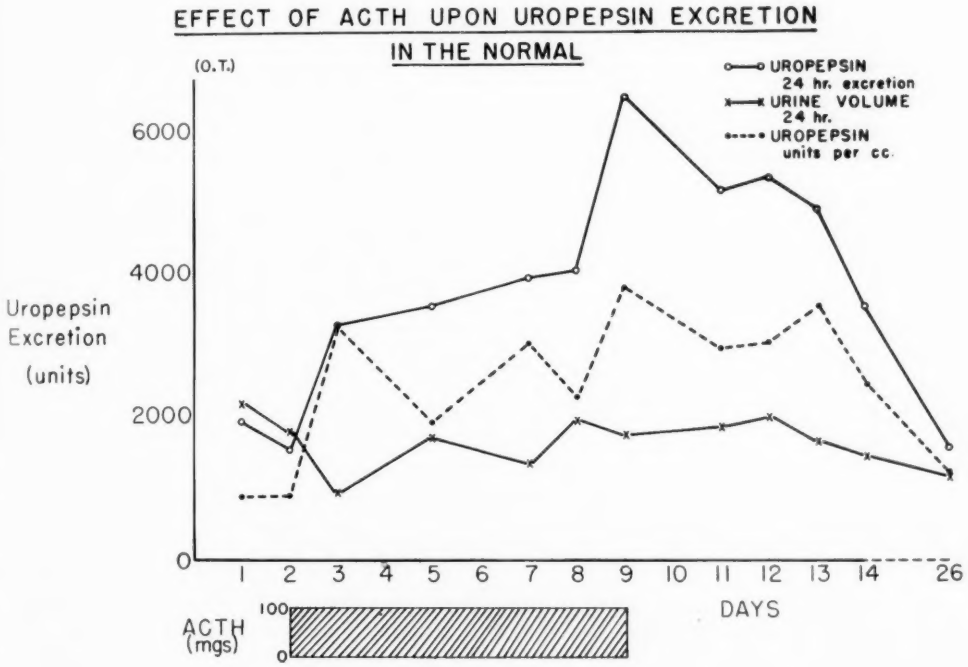

Fig. 1.

That the increased uropepsin excretion was not a function of urine volume may be seen in Fig. 1. There was no consistent change in urine volume during the administration of ACTH in this patient, and the twenty-four hour excretion of uropepsin was not affected significantly by urine volume prior to, during, or following discontinuation of the ACTH.

When the urine volume decreased, there was an increase in concentration of uropepsin resulting in an elevated twenty-four hour excretion. This was noted on the first day following the administration of ACTH, when the urine volume fell from 1,832 to 960 cubic centimeters. At that time there was a con-

TABLE II. THE EFFECT OF ACTH UPON UROPEPSIN EXCRETION

PATIENT	MEAN UROPEPSIN (UNITS/24 HR.)			MEAN UROPEPSIN (UNITS/C.C.)			MEAN URINE VOLUME PER 24 HOURS (C.C.)			ACTH ADMINISTERED (MG.)	DAYS OF THERAPY
	BEFORE ACTH	DURING ACTH	AFTER ACTH	BEFORE ACTH	DURING ACTH	AFTER ACTH	BEFORE ACTH	DURING ACTH	AFTER ACTH		
1. O. T.	1,725	4,100	1,500	.97	2.8	1.2	1,780	1,470	1,250	700	7
2. A. D.	4,200	9,860	3,170	2.1	4.3	2.7	1,600	2,350	1,130	1,080	20
3. O. R.	6,500	10,500	5,600	4.2	3.7	2.2	1,540	2,800	2,750	980	21
4. O. D.	3,930	6,700	3,800	6.1	6.3	3.8	620	1,140	1,000	2,770	36
5. T. R.	2,900	4,700	2,900	1.8	1.4	1.2	1,600	3,100	2,400	880	22
6. B. O.		5,600	2,400		6.6	2.6		870	950	1,070	21
7. G. R.	3,180	5,300	2,700	3.1	4.4	2.8	1,125	1,200	1,100	1,040	20
8. B. U.	1,080	2,680	1,350	1.3	1.9	.8	850	1,620	1,800	240	4
9. B. R.	1,006	2,000	990	.66	1.9	.9	1,605	1,111	1,790	200	7
10. K. I.	1,900	5,060		1.4	3.2		1,360	1,656		340	8
11. N. I.	1,600	6,800	1,990	1.5	3.5	1.7	1,110	2,600	1,070	720	18

comitant increase in uropepsin concentration from .97 unit to 3.2 units per cubic centimeter, resulting in an over-all increase in the twenty-four hour excretion.

When the urine volume was increased, the concentration of uropepsin was diminished, but still remained above the control level, so that the total uropepsin excretion was elevated. This was demonstrated (Fig. 1) when the urine volume increased to 1,580 c.c. on the third day of ACTH administration. The uropepsin concentration fell to 1.9, which was twice the concentration of the control. The resulting twenty-four hour excretion of uropepsin thus remained consistently elevated.

Elevated Uropepsin Excretion.—Patients with active peptic ulcer frequently excrete increased amounts of uropepsin, usually exceeding 3,000 units in twenty-four hours. In such cases, the administration of ACTH resulted in an increase of uropepsin that was somewhat delayed but equally impressive in magnitude. The twenty-four hour uropepsin excretion in Patient A. D. (Fig. 2) averaged 4,200 units during the control period. Upon the administration of 40 mg. of ACTH daily, the uropepsin excretion increased to 10,569 units upon the eighth day, 13,680 units on the fourteenth day, and 16,740 units on the twenty-second day, then fell precipitously to 10,200 units when the ACTH was discontinued, and gradually returned to its control level eight days later. It is of interest that six days of ACTH administration were necessary before the uropepsin excretion was increased significantly, although it should be emphasized that this patient received 40 mg. of ACTH daily instead of 100 mg. daily.

The concentration of uropepsin increased from an average of 2.1 units per cubic centimeter during the control period to 5.7 units on the eighteenth day of ACTH administration and returned to its initial level after the ACTH was discontinued.

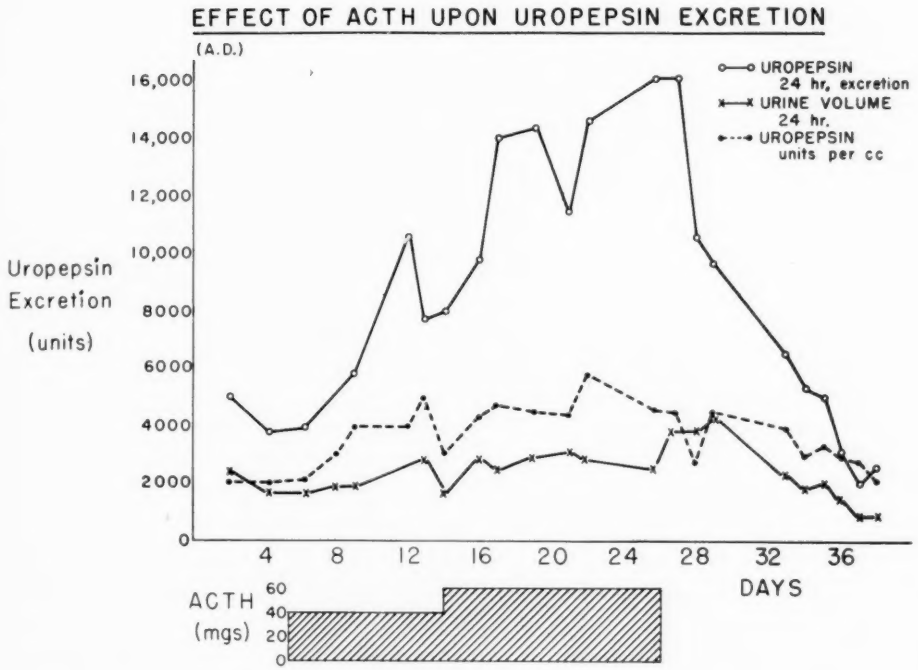
EFFECT OF ACTH UPON UROPEPSIN EXCRETION
(A.D.)
UROPEPSIN 24 hr, excretion
URINE VOLUME 24 hr.
UROPEPSIN units per cc
Uropepsin Excretion (units)
16,000
14,000
12,000
10,000
8000
6000
4000
2000
0
4 8 12 16 20 24 28 32 36
DAYS
ACTH (mgs)
60
40
20
0

Fig. 2.

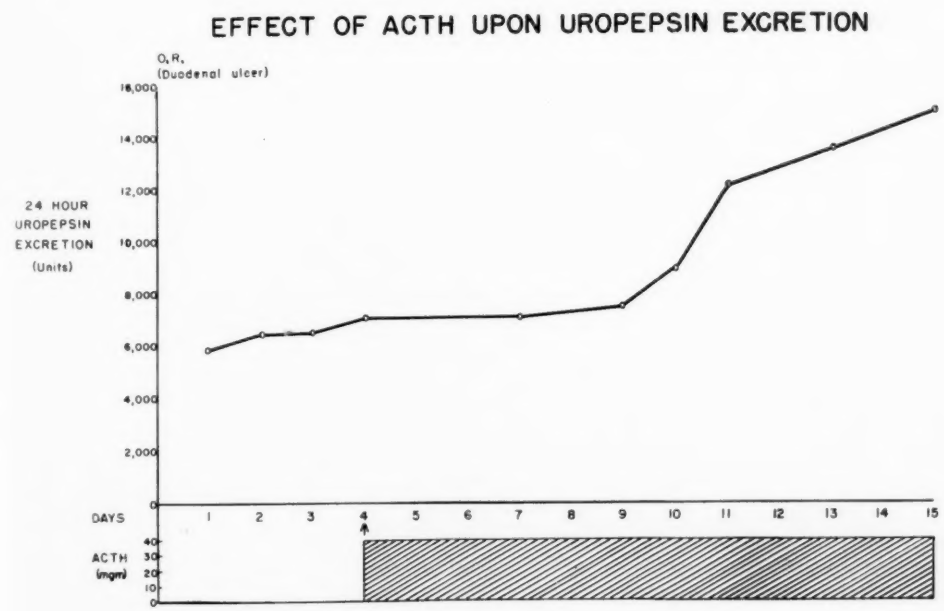
EFFECT OF ACTH UPON UROPEPSIN EXCRETION
O.R.
(Duodenal ulcer)
24 HOUR UROPEPSIN EXCRETION (Units)
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
DAYS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
ACTH (mgm)
40
30
20
10
0

Fig. 3.

The concentration of uropepsin may remain high in spite of an increase in the twenty-four hour urine volume. On the twenty-second day of ACTH administration a marked diuresis occurred, and the urine volume rose to 3,600 c.c.; the concentration of uropepsin at this point remained elevated at 4.5 units per cubic centimeter, resulting in a twenty-four hour excretion of 16,740 units. The coincidence of large urine volumes with greatly increased urine concentrations indicated a marked increase in total uropepsin production. Small urine volumes were associated frequently with an increased concentration of uropepsin, resulting in an unaltered total excretion.

The delayed response to ACTH, which occurred in patients with an initially high uropepsin excretion, is demonstrated in Fig. 3 (Patient O. R.) in which the control twenty-four hour excretion varied from 5,650 to 6,864 units. There was no increase of the twenty-four hour excretion in this patient upon the administration of 40 mg. ACTH daily until the seventh day, when the excretion rose to 12,900, and it reached its peak of 15,228 on the eleventh day. This is in contrast to the prompt response to 100 mg. of ACTH daily observed in the normal subject. The twenty-four hour excretion fell promptly to its control level three days after the ACTH was discontinued.

It may be noted from Table II that the concentration in units per cubic centimeter fell as the urine volume increased from 1,540 to 2,800 cubic centimeters. In spite of this, however, there was an over-all increase in the twenty-four hour excretion.

Diminished Uropepsin Excretion.—Similar increases in uropepsin excretion were observed in patients with low levels or complete absence of urinary uropepsin. Patient A. L. (Fig. 4) with gout and a mild atrophic gastritis, visualized gastroscopically, excreted no uropepsin. Given 50 mg. ACTH for two days, the patient excreted 2,800 units during the first twenty-four hours and 4,200 units the second day. The level promptly returned to zero after discontinuation of ACTH. The identical uropepsin response to ACTH was again produced three days later when ACTH therapy was reinstituted, and the return to zero was again prompt when ACTH was discontinued.

Gastric Cancer: In some instances of gastric cancer, uropepsin may be absent from the urine. In one such patient (Patient J. A., Fig. 5), the administration of 50 mg. ACTH produced a uropepsin excretion of 1,000 units in twenty-four hours. Uropepsin was once again absent in the urine following discontinuation of ACTH.

Impaired or Absent Gastric Secretory Function.—

Pernicious Anemia With an Atrophic Gastric Mucosa: Patients with pernicious anemia do not excrete uropepsin.[5, 6, 7] In Patient W. C. (Fig. 6), with diffuse gastric atrophy of pernicious anemia, demonstrated at gastroscopy, there was no uropepsin in the urine and no response to ACTH.

Total Gastrectomy: Following complete gastrectomy, the uropepsin level fell to zero (Fig. 7). This was demonstrated in a patient who had undergone a total gastrectomy because of an intraperitoneal chronic abscess and ulcer high

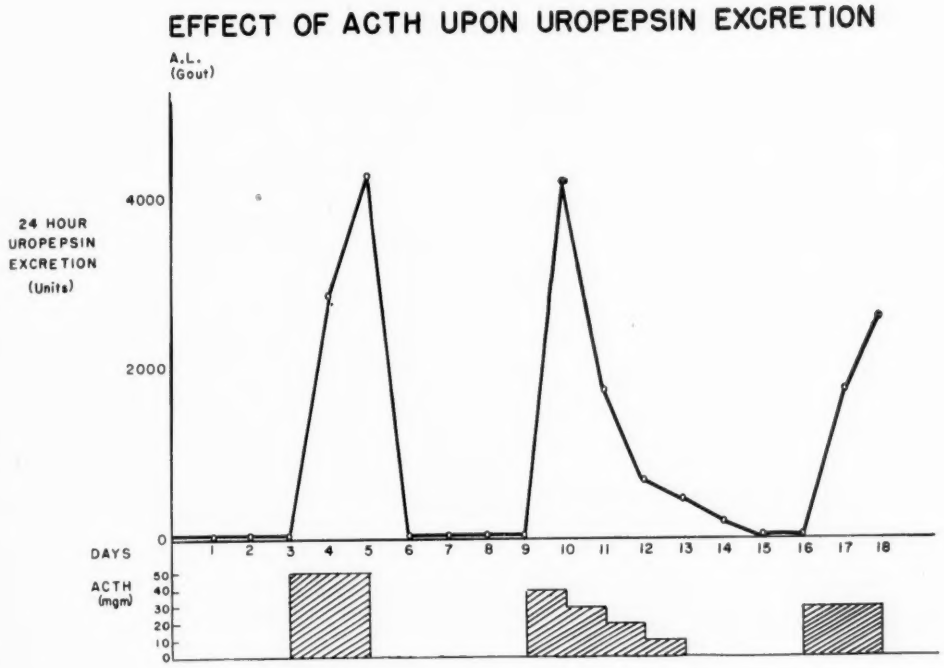
EFFECT OF ACTH UPON UROPEPSIN EXCRETION
A.L.
(Gout)
24 HOUR
UROPEPSIN
EXCRETION
(Units)
4000
2000
0
DAYS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
ACTH
(mgm)
50
40
30
20
10
0

Fig. 4.

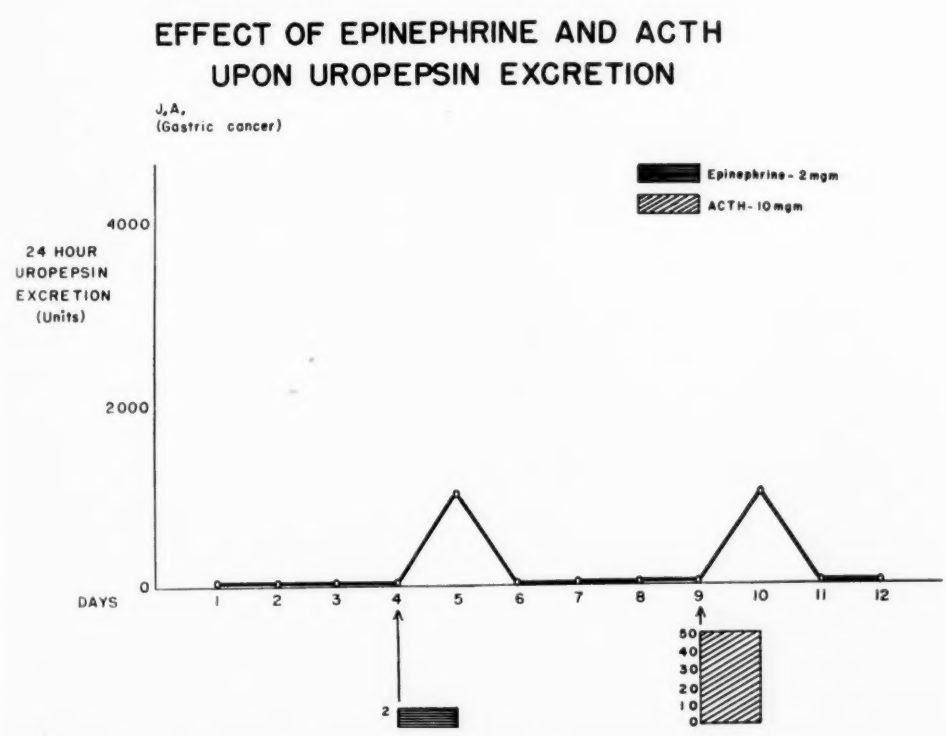
EFFECT OF EPINEPHRINE AND ACTH
UPON UROPEPSIN EXCRETION
J.A.
(Gastric cancer)
Epinephrine - 2 mgm
ACTH - 10 mgm
24 HOUR
UROPEPSIN
EXCRETION
(Units)
4000
2000
0
DAYS
1 2 3 4 5 6 7 8 9 10 11 12
2
50
40
30
20
10
0

Fig. 5.

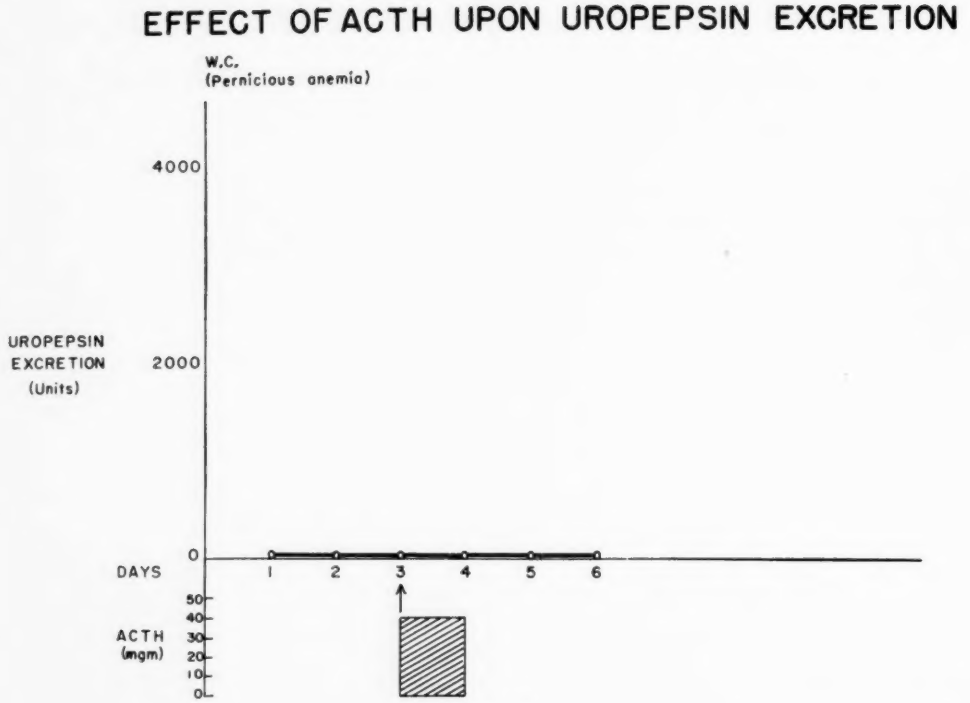
EFFECT OF ACTH UPON UROPEPSIN EXCRETION
W.C.
(Pernicious anemia)
4000
2000
0
UROPEPSIN
EXCRETION
(Units)
DAYS
1
2
3
4
5
6
50
40
30
20
10
0
ACTH
(mgm)

Fig. 6.

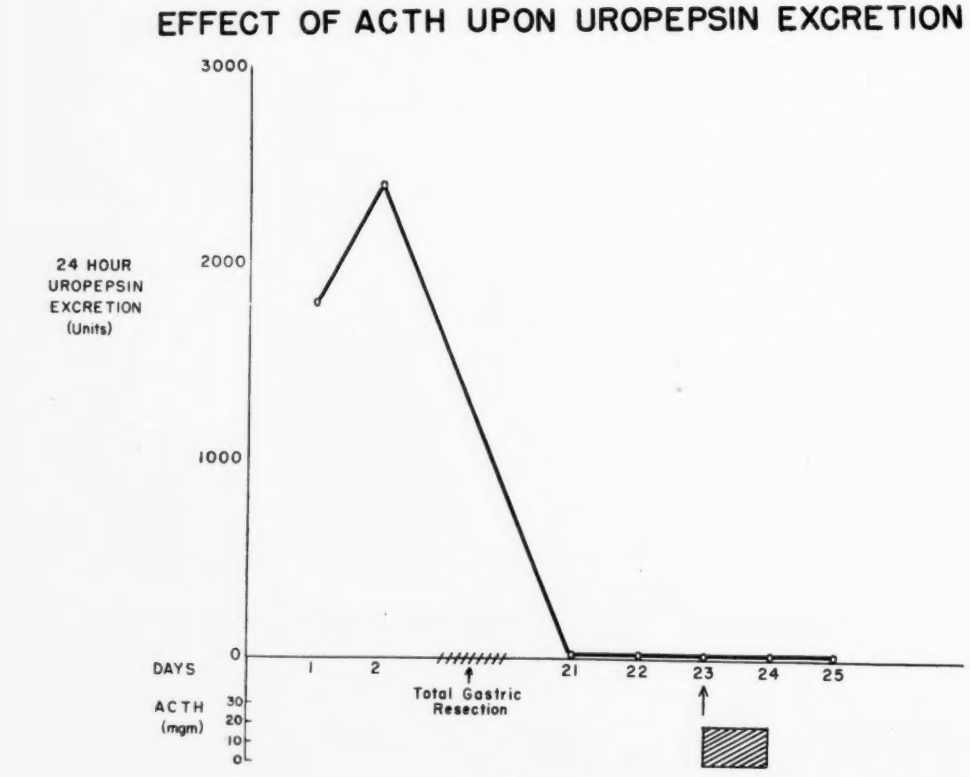
EFFECT OF ACTH UPON UROPEPSIN EXCRETION
3000
2000
1000
0
24 HOUR
UROPEPSIN
EXCRETION
(Units)
DAYS
1
2
21
22
23
24
25
Total Gastric
Resection
30
20
10
0
ACTH
(mgm)

Fig. 7.

on the lesser curvature of the stomach. The twenty-four hour uropepsin excretion prior to surgery was 1,800 to 2,300 units. After total gastric resection, the level fell to zero. Administration of ACTH over a period of two days thereafter failed to stimulate any excretion. The intact gastric mucosa appears to be essential for the uropepsin response to ACTH stimulation.

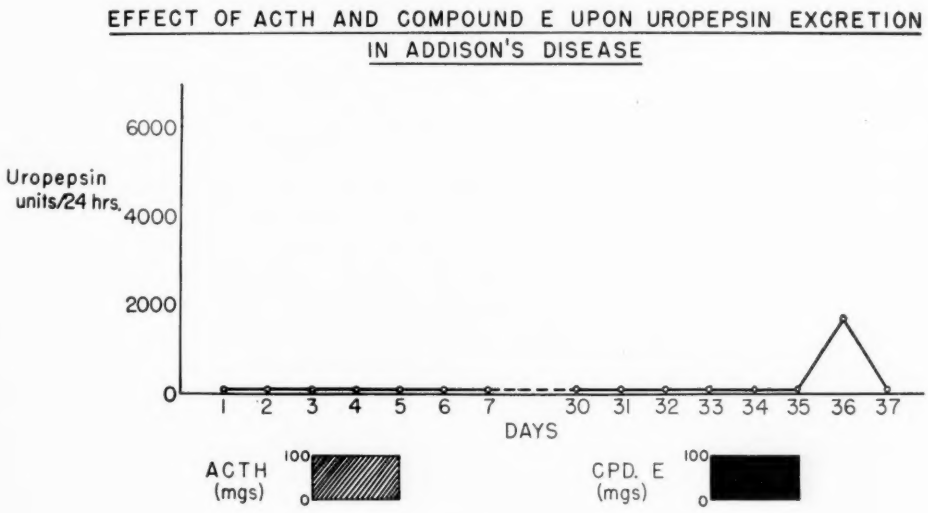

Fig. 8.

Adrenal Insufficiency.—Patients with adrenal insufficiency excrete very low levels of uropepsin, usually under 500 units. The administration of 100 mg. ACTH daily for two days failed to stimulate excretion of uropepsin, the level remaining at zero (Fig. 8). When 100 mg. of compound E were administered, the uropepsin excretion rose to 1,700 units within seventy-two hours.

TABLE III. EFFECTS OF ACTH UPON PEPSIN AND UROPEPSIN

	CONTROL PERIOD			ACTH ADMINISTRATION (10/18/49 TO 11/27/49)				RECOVERY PERIOD	
	DATE								
	10/10	10/14	10/18	10/25	11/1	11/8	11/15	12/2	12/13
Gastric pepsin (units/c.c.)	135	153	170	840	430	580	369	200	200
Uropepsin (units/24 hr.)	3,900	4,450	4,200	7,400	4,300	13,000	14,000	4,500	4,300
Dose of ACTH	0	0	0	100	100	100	40	0	0

Pepsin and Uropepsin Response to ACTH.—In one patient treated with 100 mg. doses of ACTH for thirty-six days, there was an increase in gastric juice pepsin which paralleled the elevation in urinary uropepsin (Table III). The gastric pepsin rose from control levels of 135 to 170 units per cubic centimeter to 580 to 840 units, and the uropepsin increased from the control titer

of 3,900 to 4,450 units to 13,000 to 14,000 units. When ACTH was discontinued, both gastric pepsin and urinary uropepsin returned to normal.

DISCUSSION

The administration of ACTH results in a marked increase in uropepsin excretion and concentration in the urine. In the present studies, ACTH produced a mean increase of 126 per cent in the twenty-four hour uropepsin excretion and a mean elevation of 55.6 per cent in uropepsin concentration.

The uropepsin response to ACTH administration depends upon a functioning gastric gland. Following total gastrectomy, the uropepsin excretion in the urine falls to zero and the usual response to ACTH is no longer present. In the presence of pernicious anemia and atrophy of the gastric mucosa demonstrated gastroscopically, there is essentially no uropepsin in the urine, and there is no response to ACTH administration, in spite of presumably normal adrenal function. The increase in gastric pepsin, paralleling the increase in uropepsin excretion following a prolonged course of ACTH, suggests that ACTH stimulates pepsinogen production.

The effect of ACTH upon uropepsin excretion is mediated through the adrenal gland and is not directly upon the stomach. This is evident from the studies upon the patient with Addison's disease. In the absence of adrenal function, there is essentially no uropepsin in the urine and, further, there is no increase following ACTH administration. A normal adrenal gland is, therefore, essential for the uropepsin response to ACTH. The presence of free acid and pepsin after stimulation with caffeine in this patient displays a functioning gastric mucosa. The administration of compound E, moreover, with a subsequent rise in uropepsin excretion to 1,700 units after seventy-two hours, reveals the ability of the stomach to respond directly to one of the adrenal hormones.

During ACTH administration, the increased uropepsin excretion is not affected significantly by variations in urine volume; an increase of 55 per cent in the mean uropepsin concentration, together with a similar mean rise in urine volume, indicates a true increase in the twenty-four hour excretion. With marked diuresis, the concentration of uropepsin remains unchanged or is lowered only moderately in proportion to the increase in urine volume, resulting in an over-all increase in the twenty-four hour output.

The twenty-four hour excretion of uropepsin is relatively constant from day to day in this series, despite variations in urine volume of 300 per cent (Table I). Thus, an increased urinary output is accompanied by a decrease in uropepsin concentration, and a diminished urinary volume by an increased uropepsin concentration, so that the total twenty-four hour uropepsin excretion remains unchanged.

The increased uropepsin excretion after ACTH administration does not appear to reflect a lowered renal threshold. In the first instance, patients with an initially high uropepsin excretion exhibit a response to ACTH which is

frequently delayed for three to four days (Fig. 3), while patients with a low uropepsin level prior to ACTH manifest a large increase in uropepsin excretion within twenty-four hours. Second, the gradual return to normal over a period of several days after ACTH is discontinued is also incompatible with a lowered threshold effect. Finally, the increase in gastric juice pepsin with a parallel increase in urinary uropepsin after ACTH (Table III) is good evidence of gastric gland stimulation rather than renal threshold effect.

Since the effect of ACTH upon the stomach is not a direct pharmacologic one, it is apparent that the stomach is capable of responding to an endocrine stimulus originating in the adrenal.

Although ACTH is able to stimulate uropepsin excretion to levels sometimes observed in patients with active peptic ulcer, there is no evidence as yet that this mechanism plays any role in the pathogenesis of this disease. Whether the delayed response to ACTH in these patients denotes a chronic alarm reaction remains to be determined.

Additional evidence is submitted that the reported effects of ACTH upon a legion of disease processes may be accomplished through such changes in enzyme systems as have here been described.

CONCLUSIONS

ACTH injected into experimental subjects produces an immediate and marked increase in the twenty-four hour excretion of uropepsin.

A functioning gastric gland is necessary for this response. The effect of ACTH upon uropepsin excretion is abolished by total gastric resection and is absent in patients with pernicious anemia and gastric atrophy.

The effect of ACTH upon uropepsin excretion is mediated through the adrenal gland. Patients with adrenal insufficiency excrete no uropepsin in the urine and do not respond to ACTH stimulation. The administration of compound E, however, results in an increase in uropepsin excretion.

Evidence is presented indicating that ACTH stimulates gastric gland activity by way of the adrenal gland to produce an increase in gastric juice pepsin as well as urinary uropepsin.

These studies indicate that the gastric gland responds to adrenal hormones.

The authors wish to thank Dr. George W. Thorn and Dr. Peter Forsham for their interest and cooperation during the course of these studies. The assistance of Dr. Carlyle F. Stout and Dr. Theodore Bayles of the Robert Breck Brigham Hospital is acknowledged gratefully. We are grateful to Dr. John R. Mote of the Armour Laboratories, Chicago, Ill., for providing the ACTH used in these studies.

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TOLERANCE TO THE ACTION OF ENDOTOXINS OF ENTERIC BACILLI IN PATIENTS CONVALESCENT FROM TYPHOID AND PARATYPHOID FEVERS

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MINUTE quantities of purified somatic or O antigens (i.e. endotoxins) isolated from typhoid, paratyphoid, or dysentery bacilli, when injected intravenously into man or animals, provoke physiologic reactions, including malaise, chills and fever, which are similar to those observed during the course of natural infections produced by these same organisms.[1, 2] Furthermore, the injection of these toxic somatic antigens in rabbits produces pathologic lesions, which are similar in many respects to the lesions seen in infection with *Salmonella typhosa* in man, suggesting that the latter may be related to the action of these toxic somatic antigens.[3] This possibility is further supported by the report that somatic antigen has been detected in the circulating blood early in the course of typhoid fever.[4] These observations strongly suggest that the somatic endotoxins play an important role in the production of the physiologic and pathologic changes that accompany the enteric fevers.

Other investigations have shown that, in both man and animals, tolerance to the pyrogenic effect of any of these endotoxins develops after repeated injections.[1, 5, 6, 7] Tolerance for any one endotoxin was found to be independent of circulating homologous antibody and to be effective against the toxic action of immunologically distinct somatic endotoxins from other enteric bacilli.[6, 7] Previous studies of this kind of tolerance have been made in individuals who had received repeated doses of enteric endotoxins for experimental purposes, but this phenomenon has not been investigated extensively in relation to naturally occurring infections with enteric organisms such as typhoid and paratyphoid fevers. This report presents results of tests of the pyrogenic response following the injection of purified somatic antigens in patients recovering from enteric fevers. Some of these observations were briefly summarized in a preliminary abstract.[8]

MATERIALS AND METHODS

Antigens.—The somatic antigens of *Salmonella typhosa* and *Shigella dysenteriae* (Shiga)‡ were prepared by techniques described in a previous report.[7] The stock solutions of antigens in distilled water were diluted in pyrogen-free physiologic saline solution. Before

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‡Obtained through the kindness of Dr. Manson Meads.

use, bottles used for storing the antigens and the needles and syringes employed were thoroughly washed, rinsed with distilled water, autoclaved at 15 to 20 pounds pressure for at least twenty minutes, and finally dried for fifteen minutes at 150° C.

Selection of Patients.—All typhoid and paratyphoid patients tested, unless otherwise identified, were native Egyptians from the wards of the Abbassia Fever Hospital in Cairo, Egypt. The diagnosis was substantiated by isolation of *S. typhosa* or *S. paratyphi* from the blood. Ages of patients varied from 13 to 34 years, the majority being between 17 and 26 years of age. There was a total of twenty-two patients with typhoid fever and fourteen with paratyphoid A infection; nineteen of the former and ten of the latter group were male patients. The severity of the illness in each case was graded from 1+ (mild) to 4++++ (very severe). This was based mainly on the degree of prostration or toxicity, as well as the duration and height of the fever. Each patient was checked by one of us as to history, physical examination, and urine and blood studies before the antigens were administered. In addition, at least three urine and stool cultures were taken to exclude any possible coexisting diseases that might have had an influence on the febrile response.

Control subjects comprised seventeen adult Egyptian men, all inmates of Abu Zabal prison, near Cairo. Their ages ranged from 20 to 40 years, the majority being 22 to 32 years of age. These men were evaluated by one of us as to their freedom from disease by history, physical examination, urinalysis, hematologic examinations, thick blood smears for malaria, Kahn tests for syphilis, urine and stool cultures and agglutination tests for Brucella, Proteus OX_{19}, *S. typhosa*, and *S. paratyphi*. In addition, four hourly rectal temperatures were taken at least twenty-four hours before they were injected with the antigens to aid in the exclusion of intercurrent infections.

Infestation with *Schistosoma haematobium* and intestinal parasites was undoubtedly present in the great majority of typhoid and paratyphoid fever convalescents as well as in individuals comprising the control group. However, in the absence of significant clinical manifestations of parasitic infection in any of the patients included in this report, it was felt that both the convalescent and control groups were suitable for this particular study.

In addition to the tests mentioned, tests for tolerance also were carried out in several cases of bacillary dysentery and pneumococcal lobar pneumonia in native Egyptians, as well as in one case each of tularemia, gonococcal arthritis, bacillary dysentery, and Salmonella gastroenteritis from the Boston City Hospital and the University of Michigan Hospital. Three patients convalescent from typhoid fever, who were hospitalized in these two institutions, were also included.

Administration of Antigens.—Each dose was injected into the antecubital vein in a volume of 0.25 to 1.0 cubic centimeter. In the convalescent patients, the injections were begun only after the rectal temperatures had been 100° F. or below for from four to six days. The following schedule of doses was used:

First day, 5 μg of typhoid antigen.

Second day, 1 μg of Shigella antigen.

Third day, 25 μg of typhoid antigen.

Fourth day, 5 μg of Shigella antigen.

The majority of subjects were tested with this four-dose schedule on successive days, but a few of the convalescents received only the 5 μg dose of typhoid antigen.

Rectal temperatures and pulse rates were charted just before the injection and at hourly intervals thereafter until the temperature fell below 100° F. for two or more hours. The febrile response is expressed in terms of fever units, considering each degree above 100° F. for one hour as 1 unit.

At varying intervals after the first period of testing, certain of the enteric fever convalescents were tested a second and occasionally even a third time. Individuals in the control group were also tested a second time. Before each subsequent test, patients and control subjects were questioned about intercurrent illnesses, examined, and stool and urine cultures were taken to rule out any influence of extraneous illnesses.

RESULTS

Febrile Response to Antigens in Control Group of Patients.—Seventeen healthy male subjects were tested in the manner described. Reactions consisting of headache, chilly sensations or frank shaking chills, generalized muscular aching, and occasional vomiting were observed to begin within thirty to ninety minutes after the injection of antigen. The temperature showed a rise to peak levels within two to four hours, with a more gradual return to normal in about eight hours.

Only one of the seventeen control subjects tested failed to respond with a fever above 100° F. to the first dose of 5 μg of purified typhoid antigen. The remaining sixteen subjects had febrile responses of from 2.6 to 8.8 fever units with more than half showing responses of 5 or more fever units. The average for the entire group of seventeen controls was 4.9 fever units. A 1 μg dose of Shigella antigen given on the second day produced febrile responses ranging from 3.3 to 11.1 fever units, with an average of 7.3 fever units. Twenty-five micrograms of typhoid antigen given on the third day produced a greater variation in febrile reactions than was seen with the first two test doses, ranging from 5.6 to 30.6 fever units, with an over-all average of 12.5. With this dose, fifteen of the seventeen subjects exhibited responses ranging from 7.4 to 14.8 fever units.

On the fourth day febrile responses to 5 μg of Shigella antigen ranged from 4.4 to 23.0, with an average of 13.8 fever units. The subjects showed greater variation in febrile response to this fourth dose of antigen than to any of the preceding doses. Several showed a similar or even less severe reaction to this 5 μg dose than that obtained earlier with only one-fifth of this quantity of Shigella antigen. These observations may indicate the appearance of tolerance acquired as a result of the successive test injections.[2]

The results of pyrogen tests and serum agglutination titrations made on the control subjects are listed in Table I. These data are compared with results obtained in other groups in Table V.

Twelve of the seventeen control subjects were retested with an identical schedule of doses seventeen weeks after the initial testing. Results of these repeat observations are included in Table I. Typhoid Vi antibody titers were also determined before the second series of tests (Table I).

Marked resistance to the pyrogenic action of the antigens was observed in the control subjects at the time of the second testing, which in some instances was manifested with the first dose and increased with each succeeding dose. In only about half of the control group was the febrile response comparable to that obtained after the first test injection of the previous series. In interpreting the results of this second test for tolerance, in both the control and the convalescent group of patients, it should be pointed out that the first dose of the antigen is the most significant value to consider. The influence of the phenomenon of induced resistance which follows repeated injections of antigens becomes greater as consecutive injections are administered, and tolerance, once established, rapidly reappears with subsequent injections of antigen.[6, 7]

TABLE I. FEBRILE RESPONSE TO INJECTIONS OF TOXIC SOMATIC ANTIGENS IN CONTROL SUBJECTS

SUBJECT	SHIGELLA AND TYPHOID AGGLUTININ TITERS*			AGE	WEIGHT (LB.)	FEBRILE RESPONSE TO ANTIGENS				INTERVAL (WK.)	TYPHOID AND SHIGELLA AGGLUTININ TITERS				FEBRILE RESPONSE TO ANTIGENS			
	T-O	T-H	S-O			T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)		T-VI	T-O	T-H	S-O	T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)
						FEVER UNITS									FEVER UNITS			
1	<20	40	<20	25	150	4.2	6.0	12.8	4.4	17	5	320	40	<20	1.2	1.4	3.8	1.2
2	<20	20	<20	40	117	6.0	6.8	12.2	8.0	17	<5	160	20	<20	5.2	4.0	3.6	8.0
3	<20	20	<20	35	145	3.2	5.0	11.8	7.0	17	<5	320	20	<20	2.6	0.4	3.2	1.8
4	<20	<20	<20	28	141	6.6	11.1	9.8	19.0	17	<5	320	<20	20	1.8	0.6	1.8	0.6
5	<20	<20	<20	28	130	2.6	9.4	12.6	18.6	17	<5	160	<20	40	2.8	2.8	1.4	2.0
6	<20	<20	<20	30	145	8.8	9.2	13.8	14.6	17	40	160	<20	20	5.8	4.2	3.0	3.6
7	<20	<20	<20	27	130	4.5	3.3	10.4	12.0	17	<5	160	<20	20	5.6	0.8	4.8	0.0
8	<20	<20	<20	25	134	5.0	4.2	5.6	11.0	17	<5	160	<20	20	0.0	1.2	1.4	0.0
9	<20	80	<20	22	128	6.4	6.6	10.0	14.2	17	<5	160	20	40	0.0	0.0	1.0	1.0
10	<20	160	<20	25	123	7.6	9.2	30.6	23.0	17	<5	160	80	20	3.0	0.0	3.8	1.8
11	<20	20	<20	28	132	0.0	6.8	8.4	10.4	17	10	160	<20	<20	0.4	1.0	0.4	0.0
12	<20	<20	<20	21	130	3.4	10.2	14.8	21.2	17	<5	160	<20	40	2.6	1.2	4.2	4.6
13	<20	<20	<20	32	132	6.2	5.6	14.0	6.6									
14	<20	<20	<20	25	141	5.2	9.6	13.8	20.0									
15	<20	20	<20	20	119	6.4	9.2	12.6	15.2									
16	<20	40	<20	29	143	3.0	7.6	11.2	17.8									
17	<20	20	<20	35	158	4.6	4.2	7.4	11.4									
Average						4.9	7.3	12.5	13.8						2.6	1.5	2.7	2.0

T, Typhoid; S, Shigella.

*Expressed as the reciprocal.

Febrile Response to Antigens in Convalescent Typhoid Fever Patients.—Twenty-two Egyptian patients convalescent from typhoid fever (nineteen male and three female subjects) were tested on successive days in the manner described.

Five micrograms of typhoid antigen gave febrile reactions which varied between 0.0 and 10.8 fever units, with an over-all average of 2.5 for the entire group. Fifteen of the twenty-two convalescents had responses of 2.0 units or less, and only three showed responses of more than 5.0 fever units.

Subsequent tests were carried out on only seventeen of the twenty-two typhoid fever patients. On the second day 1 μg of Shigella antigen gave responses which varied from 0.00 to 9.0 fever units, with an over-all average of 3.8 fever units for all of the seventeen patients. Twenty-five micrograms of typhoid antigen gave febrile responses ranging from 0.0 to 13.0, and an average of 4.7 fever units. The 5 μg dose of Shigella antigen produced responses ranging from 1.2 to 22.2 fever units, with an average response of 11.4 fever units.

The results of the tests in the typhoid convalescent group are given in Table II and in summary form with other data in Table V. Typhoid flagellar and somatic antibody titers as well as *S. dysenteriae* and typhoid Vi agglutinin titers on sera from these persons are listed in Table II.

Eleven typhoid patients were retested after intervals of from eleven to twenty-eight weeks following the first series of injections. In addition, three of these patients were tested a third time, nine to thirteen weeks later. The results are given in Table II.

As can be seen from Table II there was no consistent correlation between the resistance observed to the pyrogenic effect of the antigens and the titers of homologous antibody demonstrable in the sera of the patients. Those pations who had very severe or severe illnesses tended to exhibit the most marked resistance to all or at least the first three of the four antigen doses (e.g. Patients 19, 20, 23, and 29). However, Patient 26 is a notable exception to this tendency.

Of greatest significance in the consideration of the results of these tests is the fact that as a group the convalescent typhoid fever patients demonstrated a greater tolerance for the pyrogenic action of the somatic endotoxins than did the normal control subjects. This was true not only for the typhoid antigen but also for the 1 μg dose of Shigella antigen. The fourth injection of 5 μg of Shigella antigen did not reveal any significant differences in the response of the typhoid convalescents and normal controls. However, this latter test dose was the last one administered and the three previous injections of antigen may have established some degree of tolerance in the control group since previous work has shown that repeated injections of these antigens in the normal person results in the development of tolerance to their toxic effects.[2, 7]

In considering the results of the repeated tests in the various convalescents, it can be seen that, with the passage of time, there was a tendency toward loss of resistance. This was especially striking with the initial injection of antigen of each series. However, resistance rapidly reappeared with subsequent antigen injections. Decrease of resistance to the first test dose did

TABLE II. FEBRILE RESPONSE TO INJECTION BY TOXIC SOMATIC ANTIGENS IN CONVALESCENT TYPHOID PATIENTS

PATIENT	SEVERITY	TYPHOID AND SHIGELLA AGGLUT. TITERS*			FEBRILE RESPONSE TO ANTIGENS				INTERVAL (WK.)	TYPHOID AND SHIGELLA AGGLUT. TITERS			FEBRILE RESPONSE TO ANTIGENS				INTERVAL (WK.)	TYPHOID AND SHIGELLA AGGLUT. TITERS			FEBRILE RESPONSE TO ANTIGENS			
					T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)					T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)					T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)
		T-O	T-H	S-O	FEVER UNITS					T-VI	T-O	S-O	FEVER UNITS					T-VI	T-O	S-O	FEVER UNITS			
18	+++	640	320	<20	2.0	5.8	4.0	14.2	18	ND	640	640	5.6	7.8	11.4	5.0	12	<5	640	320	13.6	4.2	14.8	5.8
19	++++	640	640	<20	0.4	0.8	0.0	7.6	19	5	>1,280	<20	0.0	1.2	1.4	0.0	13	<5	640	40	0.0	0.6	0.8	0.4
20	+++	>1,280	640	<20	0.4	2.6	2.0	6.8	11	<5	640	160	1.0	0.0	2.8	1.6	10	<5	320	160	2.2	0.4	2.8	2.2
21	++	>1,280	80	<20	3.6	2.8	10.8		23	20	>1,280	640	1.0	0.8	12.0									
22	+++	<20	640	20	0.0	4.4	1.8	13.6	14	<5	80	160	0.0	0.6	6.6	6.1								
23	++++	1,280	1,280	20	0.0	3.4	3.0	1.2	18	<5	80	20	6.6	1.0	14.2	13.8								
24	++++	>1,280	640	160	2.0	4.6	5.6	8.0	14	160	320	80	2.6	0.0	5.2	1.2								
25	++	160	160	<20	5.0	1.4	8.6	17.2	26	20	320	40	7.6	5.4	5.4	5.2								
26	+	640	640	<20	0.0	0.0	0.4	6.2	28	<5	80	40	0.4	1.2	4.6	1.0								
27	++++	640	320	<20	0.4	2.8	2.6	10.8	22	ND	640	160	1.8	3.0	5.4	3.8								
28	+	320	640	<20	0.8	9.0	3.2	16.2	21	20	<20	80	6.8	3.4	8.2	5.2								
29	++++	160	640	<20	1.4	3.6	0.6	8.4																
30	++	<20	<20	<20	10.8	3.2	13.0	22.2																
31	+++	640	>1,280	<20	2.0	2.6	6.6	14.8																
32	+++	640	>1,280	<20	5.0	7.4	10.0	21.0																
33	++++	320	>1,280	<20	5.6	6.4	4.4	8.4																
34	+++	640	>1,280	<20	2.0	3.8	3.6	6.2																
35	+++	<20	320	<20	0.0																			
36	++++	80	320	<20	8.4																			
37	++++	80	320	<20	5.0																			
38	+++	160	320	<20	0.0																			
39	+++	<20	640	20	0.0																			
Average					2.5	3.8	4.7	11.4					3.0	2.2	7.0	3.8					5.3	1.7	6.1	2.8

T, Typhoid; S, Shigella.
ND, Not done.
*Expressed as the reciprocal.

not occur in every case since three patients (Patients 19, 21, and 24) exhibited no loss of resistance to the antigens fourteen to twenty-three weeks after the first series of antigen injections. Also, in the convalescent patients that were retested, there was no consistency in the time required to show loss of resistance. Furthermore, the degree of loss of tolerance after comparable intervals of time varied considerably from patient to patient.

Febrile Response to Antigens in Paratyphoid A Fever Patients.—Fourteen patients convalescing from paratyphoid A fever (ten male and four female subjects) were available for testing with the typhoid and Shigella antigens. Eleven of the fourteen patients received a full four-dose schedule and the remaining three were given the first dose only. The responses in the individual patients as well as serum antibody titers and data obtained in the repeat tests are shown in Table III. Four paratyphoid convalescents were retested at intervals of from eighteen to twenty-eight weeks after the first testing, and three of these were tested a third time after an additional interval of nine to ten weeks following the second tests. The results in this group were essentially the same as those obtained in the patients with typhoid fever.

In the paratyphoid group, as was noted previously with the typhoid patients, there was no correlation between the magnitude of febrile reaction produced by the antigens and the homologous, serum antibody titers. In contrast to the typhoid patients, there was no suggestion of a relationship between the degree of resistance to the antigens and severity of the disease in the individuals convalescent from paratyphoid fever. However, the striking feature is the fact that the convalescent paratyphoid patients showed practically the same degree of resistance to the pyrogenic effect of the typhoid and Shigella antigens as was exhibited by the patients convalescing from typhoid fever.

Febrile Response to Antigens in American Patients With Typhoid Fever and in Patients With Other Diseases.—Three cases of typhoid fever in American patients as well as one case each of tularemia, gonococcal arthritis, bacillary dysentery (*S. sonnei*), and Salmonella gastroenteritis (*S. newport*) were tested, after the patients had been afebrile for from five to ten days, with 5 μg typhoid and 1 μg of Shigella antigen on successive days. These patients were all hospitalized at the Boston City Hospital or at the University of Michigan Hospital. Three Egyptian patients with bacillary dysentery (Patients 61, 62, and 63) and two with pneumococcal lobar pneumonia (Patients 64 and 65) were tested with the four-dose schedule of antigen injections described previously. Results in this group of patients are listed in Table IV. A comparison of the febrile responses observed in this group of patients with those of the typhoid and paratyphoid A patients is furnished in Table V.

The three American patients with typhoid fever showed a definite and marked resistance to the antigens with which they were tested. In contrast, the American patients with tularemia, gonococcal arthritis, bacillary dysentery, and Salmonella gastroenteritis showed no evidence of resistance to the pyrogenic action of the test antigens. Of special interest are the reactions observed in the patient with Salmonella gastroenteritis since, in this type of salmonellosis, the organisms do not invade the blood stream. Therefore, somatic anti-

TABLE III. FEBRILE REACTIONS FOLLOWING INJECTION OF TOXIC SOMATIC ANTIGENS IN CONVALESCENT PARATYPHOID A PATIENTS

PATIENT	SEVERITY	TYPHOID AND PARA A AGGLUTININ TITERS*				FEBRILE RESPONSE TO ANTIGENS				INTERVAL (WK.)	"O" AGGLUTININ TITERS			FEBRILE RESPONSE TO ANTIGENS				INTERVAL (WK.)	"O" AGGLUTININ TITERS		FEBRILE RESPONSE TO ANTIGENS			
		A-O	A-H	T-O	T-H	T (5 $\mu\sigma$)	S (1 μg)	T (25 μg)	S (5 μg)		A-O	T-O	S-O	T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)		T-O	S-O	T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)
						FEVER UNITS								FEVER UNITS							FEVER UNITS			
40	++	80	1,280	<20	80	0.4	3.6	5.0	7.8	18	80	320	160	4.8	1.4	13.4	5.0	9	320	160	8.0	1.6	8.2	4.6
41	++	20	320	<20	<20	4.0	3.0	7.0	19.8	23	<20	1,280	640	1.6	1.8	14.2	8.4	10	640	640	4.8	0.0	5.8	9.6
42	++++	80	80	<20	1,280	0.0	3.0	4.2	7.4	25	<20	160	80	1.0	0.4	0.0	0.4	10	640	80	1.2	0.0	2.6	0.3
43	+++	40	<20	<20	<20	0.2	2.0	8.2	29.0	28	<20	<20	80	9.2	3.0	10.8	7.8							
44	±	320	1,280	80	20	0.0	2.4	3.2	12.4															
45	++	20	<20	160	80	0.0	0.8	0.4	21.8															
46	+++	20	1,280	80	40	10.0	5.2	9.8	15.0															
47	+++	640	160	40	<20	6.4	10.0	14.6	16.8															
48	++	<20	320	<20	<20	5.0	5.0	7.2	10.8															
49	+++	20	1,280	640	20	3.0	6.2	4.8	13.0															
50	±	20	<20	20	<20	1.2	0.0	4.4	2.8															
51	+	40	>1,280	<20	<20	0.6																		
52	+	<20	80	<20	<20	0.4																		
53	+	80	640	80	160	2.8																		
Average						2.4	3.7	6.3	14.2					4.1	1.6	9.6	5.4				4.7	0.5	5.5	5.0

A, Para A; T, Typhoid; S, Shigella.
Titers against *S. dysenteriae* were <20 in all patients before initial testing.
*Expressed as the reciprocal.

TABLE IV. REACTIONS FOLLOWING INJECTION OF TOXIC SOMATIC ANTIGENS IN CONVALESCENT AMERICAN TYPHOID FEVER PATIENTS AND IN PATIENTS WITH OTHER DISEASES

PATIENT	HOSPITAL	ETIOLOGY OF DISEASE		SEVERITY	DAYS AFEBRILE BEFORE TEST	TYPHOID AND SHIGELLA AGGLUT. TITERS*			FEBRILE RESPONSE TO ANTIGENS			
		ORGANISM	SOURCE			T-O	T-H	S-O	T (5 μg)	S (1 μg)	T (25 μg)	S (5 μg)
									FEVER UNITS			
54	B.C.H.	S. typhosa	Blood	++	10	128	ND	ND	0.0	1.4		
55	U.M.H.	S. typhosa	Stool	+++	10	0	2,000	ND	1.3	2.0		
56	U.M.H.	S. typhosa	None	++	5	40	1,600	ND	1.0	0.7		
57	B.C.H.	S. newport	Stool	++	7	16	ND	ND	16.2	ND		
58	U.M.H.	Past. tularensis	None	++	14	80	640	ND	5.0	2.0		
59	B.C.H.	Gonococcus	Synovial fluid	+++	7	0	ND	ND	6.0	67		
60	B.C.H.	S. sonnei	Stool	+++	7	0	ND	ND	6.0	8.2		
61	Egypt	S. paradysenteriae	Stool	++	3	20	20	<20	0.0	1.8	3.6	1.0
62	Egypt	S. paradysenteriae	Stool	++	5	320	20	<20	3.2	0.2	8.8	13.2
63	Egypt	S. paradysenteriae	Stool	++	7	80	40	<20	5.2	8.4	11.4	15.0
64	Egypt	Pneumo. Type 1	Sputum	+++	4	160	40	<20	1.2	0.0	6.8	16.6
65	Egypt	Pneumo. Type 2	Sputum	+++	4	320	<20	<20	6.4	5.2	6.0	14.0

T, Typhoid; S, Shigella; ND, not done.

*Expressed as the reciprocal. Agglutination test for Shigella was done with *S. dysenteriae* and does not represent titer of agglutinins against the patient's own organism.

gen would not be present in the circulation in quantity during the infection to stimulate the development of tolerance to its toxic effects. The results obtained in the Egyptian patients with bacillary dysentery and pneumonia are difficult to interpret. One Egyptian patient with bacillary dysentery showed complete resistance to all four doses of antigens; another was resistant to the 1 μg dose of Shigella antigen; and one of the patients with pneumonia appeared to be resistant to the smaller doses of antigens. In this regard, the high

TABLE V. SUMMARY OF FEBRILE RESPONSES IN VARIOUS GROUPS OF PATIENTS TESTED WITH TOXIC SOMATIC ANTIGENS

DOSE	GROUP	FEBRILE RESPONSE IN FEVER UNITS										AVERAGE	TOTAL NUMBER OF CASES
		0-1.9	2-3.9	4-5.9	6-7.9	8-9.9	10-11.9	12-13.9	14-15.9	16-17.9	OVER 18		
Typh. 5 μg	Typhoid (Egypt)	11	5	4	0	1	1					2.4	22
	Typhoid (U.S.)	3										0.8	3
	Para A	8	2	2	1	0	1					2.4	14
	Misc. diseases*	1	0	1	2	0	0	0	0	1		7.0	5
	Bacillary dys.	1	1	1	1							3.6	4
	Controls	1	4	5	6	1						4.9	17
Shig. 1 μg	Typhoid (Egypt)	3	8	3	2	1						3.8	17
	Typhoid (U.S.)	2	1									1.4	3
	Para A	2	5	2	1	0	1					3.7	11
	Misc. diseases*	1	1	1	1							3.5	4
	Bacillary dys.	2	0	0	0	2						4.6	4
	Controls	0	1	4	5	5	2					6.3	17
Typh. 25 μg	Typhoid (Egypt)	4	5	3	1	1	2	1				4.7	17
	Para A	1	1	4	2	2	0	0	1			6.3	11
	Controls	0	0	1	1	2	4	6	2	0	1	12.4	17
Shig. 5 μg	Typhoid (Egypt)	1	0	0	4	3	1	1	2	2	2	12.8	16
	Para A	0	1	0	2	0	1	2	1	1	3	14.2	11
	Controls	0	0	1	2	1	3	1	3	1	5	13.8	17

*These include the cases of tularemia, gonococcal arthritis, Salmonella gastroenteritis and the two cases of pneumococcal pneumonia.

typhoid O serum agglutinin titers in several of the Egyptian patients in question raise the possibility that they might have had a recent enteric infection, which would therefore throw doubt upon the significance of the diminished febrile responses obtained with the test antigens.

DISCUSSION

It is apparent that, in spite of the variations in the results obtained with individual patients, individuals convalescent from typhoid or paratyphoid fevers have a greater resistance to the pyrogenic effects of somatic endotoxins of *S. typhosa* and *S. dysenteriae* (Shiga) than do normal individuals or patients convalescing from diseases not involving an invasion of the blood stream by organisms of the Salmonella or Shigella groups. Furthermore, when the results obtained in the typhoid and paratyphoid patients were compared with those of control subjects using the statistical technique of analysis of variance,[9] it was found that there was less than one chance in a thousand that the observed differences were a matter of chance. The data indicate that tolerance to the toxic action of endotoxin of *S. typhosa* develops during the course of natural infections with this organism or with *S. paratyphi* in man. This tolerance has a broad coverage extending to the chemically similar but antigenically distinct endotoxin of *S. dysenteriae* (Shiga). The development of this tolerant state appears to be specific in that it does not appear following other infectious diseases such as tularemia, gonococcal arthritis, or pneumonia.

Certain observations by other investigators are not in entire agreement with the results reported here, but the number of patients with typhoid fever studied was small. Heyman and Beeson[10] reported pyrogenic responses to injections of typhoid vaccine in patients convalescent from a variety of acute infections that were comparable to those seen in normal individuals. Two patients with typhoid fever included in this study also gave good febrile responses to the bacterial pyrogen. Following experimental infections with *Escherichia coli* and pneumococci in rabbits, Bennett[11] failed to demonstrate any increased resistance to the pyrogenic action of killed suspensions of *S. typhosa* or *Esch. coli*. This experimental study, however, does not reproduce the type of infection seen in typhoid fever in man.

Patients with paratyphoid A fevers also demonstrated a resistance to the pyrogenic action of endotoxins of *S. typhosa* even though most of them had little or no antibody for this antigen demonstrable in their blood serum. This observation and the presence of resistance in both the paratyphoid and typhoid convalescents to the toxic action of the endotoxin of *S. dysenteriae* (Shiga), for which no antibodies could be detected in the sera of patients, clearly indicate that the tolerant state following these natural infections has the same broad coverage and independence of circulating antibody previously observed in the tolerance established by experimental methods.[2, 7] It may well be that this tolerance is due to an increased activity of the reticuloendothelial system as previously suggested by Beeson.[12]

Tolerance to pyrogenic antigens which has been induced by repeated doses is reported to be lost in rabbits after a period of about three weeks,[5] and in four to five weeks in man.[7] Attempts to determine the duration of the resistant state in this study by retesting after variable periods of time were inconclusive. In the control group of patients, tolerance persisted for as long as seventeen weeks in some persons. There was considerable variation in the duration of tolerance among the convalescent patients. In some cases there was a definite loss of tolerance after eighteen weeks, but in others tolerance apparently persisted into the twenty-third week after initial testing. Results obtained by retesting were probably complicated by the fact that a schedule of four successive test doses of increasing magnitude was employed instead of a single dose. A mechanism of rapid re-establishment or recall of tolerance by a second, third, and fourth test dose in an individual who had previously received the antigens might account for some of the inconsistency in febrile reactions found on retesting.[7] This appearance of tolerance as a result of the test injections accounts for the fact that the febrile responses of the control subjects to the fourth test injection were almost identical with those seen in the typhoid and paratyphoid convalescents (Table V).

The development of the state of tolerance may well have a role in recovery from typhoid and paratyphoid fevers. As such, it appears to be a mechanism of immunity distinct from those associated with the presence or activity of humoral antibody. A complete understanding of the mechanism of tolerance awaits further information, but its elucidation may lead to a better understanding of factors involved in recovery from infectious diseases.

SUMMARY

Patients convalescent from typhoid and paratyphoid A fevers were tested with a series of intravenous doses of purified somatic endotoxins of *S. typhosa* and *S. dysenteriae* (Shiga), and the results were compared with those obtained in a control group of healthy subjects tested in the same manner.

Definite tolerance or resistance to the pyrogenic action of toxic somatic antigens of *S. typhosa* and *S. dysenteriae* (Shiga) was demonstrated in both the typhoid and paratyphoid A convalescents when their responses were compared with those obtained in the control subjects.

Tolerance to the toxic effects of the somatic antigens was not correlated with the amount of homologous antibody demonstrable in the serum of the individuals tested.

A few patients convalescent from diseases such as bacillary dysentery, gonococcal arthritis, pneumococcal pneumonia, and tularemia were also tested with the endotoxins. On the whole, the results suggested that these diseases were not associated with the appearance of tolerance to the toxic effects of these antigens.

The possible significance of tolerance to the toxic somatic antigens in the mechanism of recovery from enteric infections is discussed.

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SPLANCHNIC BLOOD FLOW IN MAN BY THE BROMSULFALEIN METHOD: THE RELATION OF PERIPHERAL PLASMA BROMSULFALEIN LEVEL TO THE CALCULATED FLOW

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IN 1944 Warren and Brannon[1] described a safe method for catheterizing the hepatic vein in man. The following year, Bradley and co-workers[2] adopted this technique for the estimation of splanchnic blood flow by measuring hepatic extraction of bromsulfalein (BSP). Other workers used this method, but the estimated splanchnic blood flow (E.S.B.F.) has been followed for only relatively short periods.[3-6] The present report describes splanchnic blood flow over two and one-half to three hours in normal subjects. In particular, the relation of the BSP concentration in the peripheral blood to the calculated splanchnic blood flow is discussed.

METHODS AND MATERIAL

The forty-nine normal subjects had no known hepatic dysfunction.

Experimental Procedure.—Observations were made after the subjects had been at rest in bed and had fasted for twelve hours. Sodium Amytal, 0.2 Gm. by mouth, was given thirty minutes before the observations were begun.

The technique for estimating splanchnic blood flow was essentially that of Bradley and associates.[2] A branch of the right hepatic vein was catheterized under fluoroscopic control. The catheter was kept in the same position throughout and this was fluoroscopically checked at the conclusion of the observation.

A priming dose of 150 mg. BSP was given and then BSP was infused in 0.5, 0.6, or 0.7 Gm. per cent solution. The infusion was maintained at a rate of about 1 ml. per minute, using a motor-driven 50 ml. syringe, modified from the model used by Allen and co-workers.[7] The mean dose of BSP was 0.090 (0.043 to 0.156) mg. per kilogram per minute.

Blood samples were taken simultaneously from the hepatic vein catheter and through an indwelling needle in an antecubital vein. The latter was used instead of an indwelling arterial needle because of the greater ease of repeated sampling over long periods. It was found that peripheral venous and arterial blood samples, taken simultaneously, showed identical BSP concentrations.

Blood samples were taken at intervals of five to twenty minutes. After the priming dose of BSP, at least twenty minutes elapsed before the first sample was taken. If the BSP concentration of the infusion had to be altered, then another twenty-minute stabilization period was allowed before further samples were withdrawn. The observations were continued for two and one-half to three hours.

Analytical Methods: Blood samples were taken into heparinized tubes and analyzed on the same day. After an initial centrifugation, the supernatant plasma was recentrifuged. Turbid and hemolyzed samples were discarded. Two milliliters of plasma were added to 4 ml. 0.9 Gm. per cent sodium chloride. Readings were made with an Evans photoelectric

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TABLE I. ESTIMATED SPLANCHNIC BLOOD FLOW IN 49 NORMAL SUBJECTS

SUBJECT	SEX AND AGE	SURFACE AREA (SQ. M.)	WEIGHT (KG.)	BSP		E.S.B.F. (ML./MIN.)	E.S.B.F. (ML./SQ.M./MIN.)	BSP (PER CENT HEPATIC EXTRACTION)
				INFUSION RATE (MG./KG./MIN.)	PERIPHERAL LEVEL (MG./100 ML.)			
A. PERIPHERAL BSP LEVEL LESS THAN 1 MG./100 ML.								
C. M.	M, 42	1.93	76	0.043	0.48	5,260	2,720	25
C. H.	M, 49	1.72	62	0.075	0.50	3,330	1,940	47
T. R.	M, 29	1.60	50	0.099	0.50	2,410	1,500	70
E. T.	M, 37	1.79	63	0.079	0.54	2,280	1,270	77
J. J.	M, 40	1.49	50	0.091	0.62	2,570	1,720	51
M. McG.	F, 34	1.54	53	0.094	0.65	2,040	1,321	49
F. S.	M, 37	1.92	74	0.074	0.68	2,540	1,320	56
G. F.	F, 45	1.66	60	0.097	0.70	1,960	1,182	69
J. P.	M, 56	1.79	67	0.108	0.71	1,940	1,080	79
J. H.	F, 34	1.44	48	0.104	0.72	1,635	1,135	67
M. C.	F, 52	1.25	32	0.156	0.75	2,260	1,810	36
M. F.	F, 42	1.36	40	0.093	0.76	2,760	2,030	27
P. T.	M, 24	1.60	49	0.096	0.79	1,800	1,120	61
J. H.	M, 26	1.59	51	0.098	0.81	1,640	1,020	69
E. C.	M, 29	1.94	73	0.063	0.85	2,490	1,280	34
W. W.	M, 38	1.72	63	0.082	0.85	1,962	1,140	58
J. T.	M, 58	1.99	84	0.057	0.88	1,930	970	46
Mean				0.089	0.68	2,411	1,530	54.1
Standard error				0.0060	0.04	194	126	4.0
B. PERIPHERAL BSP LEVEL 1 MG./100 ML. OR MORE								
J. S.	M, 56	1.68	61	0.095	1.00	1,350	804	78
J. K.	M, 40	1.87	67	0.075	1.00	1,550	830	52
T. B.	M, 22	1.91	70	0.070	1.01	2,260	1,180	42
C. S.	M, 47	1.75	60	0.088	1.02	1,561	895	58
J. W.	M, 36	1.72	63	0.078	1.02	1,613	940	54
G. D.	M, 68	1.53	46	0.094	1.05	1,120	723	63
E. F.	F, 45	1.65	60	0.109	1.09	1,480	890	70
P. H.	M, 34	1.93	72	0.094	1.14	2,500	1,290	43
H. S.	M, 62	1.53	55	0.105	1.17	1,360	890	69
T. D.	M, 67	1.56	48	0.106	1.18	1,240	795	43
C. R.	M, 51	1.71	58	0.069	1.19	1,296	760	41
J. B.	M, 46	1.78	65	0.090	1.19	1,940	1,090	48
J. F.	F, 21	1.50	50	0.112	1.22	1,290	860	55
J. M.	M, 57	1.57	60	0.086	1.22	1,120	715	70
I. S.	M, 40	1.77	65	0.062	1.25	1,262	715	76
F. B.	M, 64	1.74	64	0.072	1.28	1,600	920	41
B. W.	M, 45	1.72	63	0.079	1.32	1,050	610	68
T. O'N.	M, 20	1.66	57	0.100	1.40	1,760	1,060	41
P. H.	M, 34	1.44	48	0.107	1.41	1,380	960	48
J. M.	M, 57	1.55	54	0.065	1.42	912	588	50
J. N.	M, 65	1.53	48	0.096	1.48	1,564	1,020	40
D. M.	M, 50	1.56	49	0.114	1.52	1,226	788	50
J. P.	M, 56	1.79	67	0.108	1.55	1,515	845	54
C. M.	M, 33	1.79	69	0.083	1.57	1,500	850	50
T. E.	M, 36	1.50	44	0.108	1.60	1,495	999	33
A. A.	M, 75	1.57	50	0.100	1.62	908	578	57
E. M.	M, 34	1.93	76	0.082	1.90	1,475	769	43
J. T.	M, 56	1.46	47	0.131	1.91	1,038	710	38
J. M.	M, 60	1.70	60	0.097	1.99	1,300	765	40
J. F.	F, 45	1.64	60	0.091	2.39	1,270	775	31
R. R.	M, 63	1.69	57	0.102	2.82	845	500	38
M. H.	M, 60	1.56	49	0.112	3.25	1,030	660	28
Mean				0.090	1.45	1,400	836	50.7
Standard error				0.00028	0.10	63	31	2.18

colorimeter and an Ilford green (625) filter. After the initial "blank" reading, a drop of 40 per cent sodium hydroxide was added and a further reading taken. The concentration of BSP in the blood was then calculated. The concentration of BSP in the solution used for infusion was determined in every experiment. The hematocrit value was determined on one of the first three hepatic venous samples, using the Wintrobe technique. The oxygen unsaturation of the hepatic vein blood was estimated on 5 ml. samples using a Haldane blood gas apparatus.[8]

Calculation.—If the infusion rate of BSP (Q) is adjusted to maintain a constant blood level (P), then Q is also the rate of removal of BSP by the liver and the Fick principle is applicable. E. S. B. F. may then be calculated by the method of Bradley and co-workers[2] according to the following formulas.

$$\text{Estimated splanchnic plasma flow} = \frac{100\ Q}{P\text{-}H}\ \text{ml./min.}$$

and

$$\text{Estimated splanchnic blood flow} = \frac{100\ Q}{P\text{-}H} \times \frac{1}{100\text{-}Ht.}\ \text{ml./min.}$$

where Q = infusion rate of BSP ml./min.

P = mg. BSP/100 ml. plasma (peripheral vein).
H = mg. BSP/100 ml. plasma (hepatic vein).
Ht. = Hematocrit value.

(Allowance was made for changing BSP values in the peripheral blood provided the rate of change was not greater than 0.0005 mg. per milliliter per minute.)

The percentage extraction of bromsulfalein was calculated.

$$\text{Per cent extraction} = \frac{P\text{-}H}{P} \times 100.$$

RESULTS

The subjects were divided into two groups depending on whether the peripheral BSP concentration was greater or less than 1 mg. per 100 milliliters. The mean infusion rate of BSP in both groups was similar.

In thirty-two subjects the peripheral BSP concentration was greater than 1 mg. per 100 ml. and the mean E.S.B.F. was found to be 836 ± 31 ml. per minute per square meter (Table I). This corresponds with values previously reported in the literature (Table II). There was no correlation between the peripheral venous BSP level and the E.S.B.F. (Fig.1).

TABLE II. COMPARISON OF PRESENT RESULTS WITH THOSE PREVIOUSLY REPORTED

AUTHOR	DATE	NUMBER OF SUBJECTS	PERIPHERAL PLASMA BSP CONCENTRATION (MG. 100 ML.)	ESTIMATED SPLANCHNIC BLOOD FLOW (ML./SQ.M.)
Bradley and co-workers	1945	23	0.94 ± 0.04*	865 ± 24 (640-1070)
Myers	1947	12	1.61 ± 0.22	850 ± 71 (600-1160)
Bondy and co-workers	1949	7	—	851 ± 71 (641-1210)
Sherlock and co-workers	1950	32	1.45 ± 0.10	836 ± 31 (500-1290)
		17	0.68 ± 0.04	1530 ± 126 (970-2720)

*Throughout this paper in the expression of ± values the standard error is used.

In seventeen subjects the peripheral venous BSP concentration was less than 1 mg. per 100 ml. and the mean E.S.B.F. was 1530 ± 126 ml. per minute per square meter (Tables I and II). There was a positive inverse correlation between peripheral venous BSP concentration and E.S.B.F. (Fig. 1).

A statistical difference ($p = 0.02$) was established between the mean E.S.B.F.'s corresponding to peripheral BSP values greater or less than 1 mg. per 100 milliliters.

Effect of Changing the Peripheral Bromsulfalein Level on Estimated Splanchnic Blood Flow.—In twenty-two subjects the peripheral BSP value showed a slow consistent rise during the two- to three-hour period of the experiment, despite the BSP infusion rate's remaining constant. The calculated splanchnic blood flow at the beginning of the observation period was compared

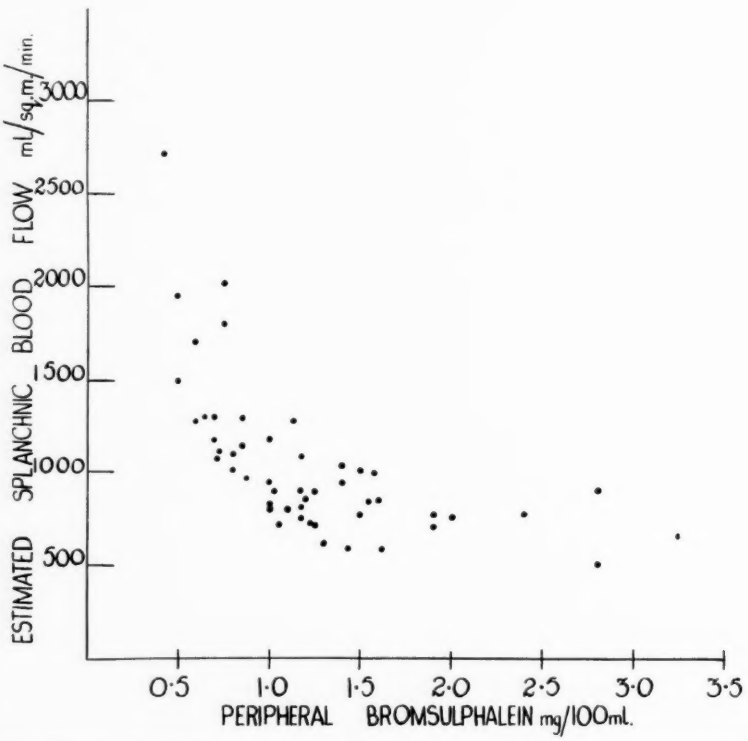

Fig. 1.—The relation of peripheral venous bromsulfalein values to estimate splanchnic blood flow.

with that at the end (Fig. 2). When the peripheral BSP value was below 1 mg. per 100 ml., an increase in peripheral BSP resulted in an apparent fall in E.S.B.F. If the initial levels were greater than 1 mg. per 100 ml., then further increase in the peripheral BSP level did not lead to any significant change in the calculated splanchnic blood flow.

In two subjects a more detailed study of the effect of changing peripheral venous BSP values was made. The results in one of these subjects are illustrated (Fig. 3). Reduction of the peripheral BSP level by altering the amount of BSP infused resulted in an apparent increase in E.S.B.F. This rise in E.S.B.F. persisted while the peripheral BSP level remained low, even when the original rate of infusion was resumed. Finally, a further "priming" dose of BSP was given and the infusion rate of BSP was again increased. With the resultant high peripheral BSP value, E.S.B.F. returned to a value comparable with those initially recorded. During the course of these observations, the subject's general condition, pulse, and blood pressure did not change. Moreover, there

was no significant change in the percentage saturation of the hepatic venous blood with oxygen. These data suggest that, despite the calculated results, splanchnic blood flow did not in fact alter significantly during the period of observation.

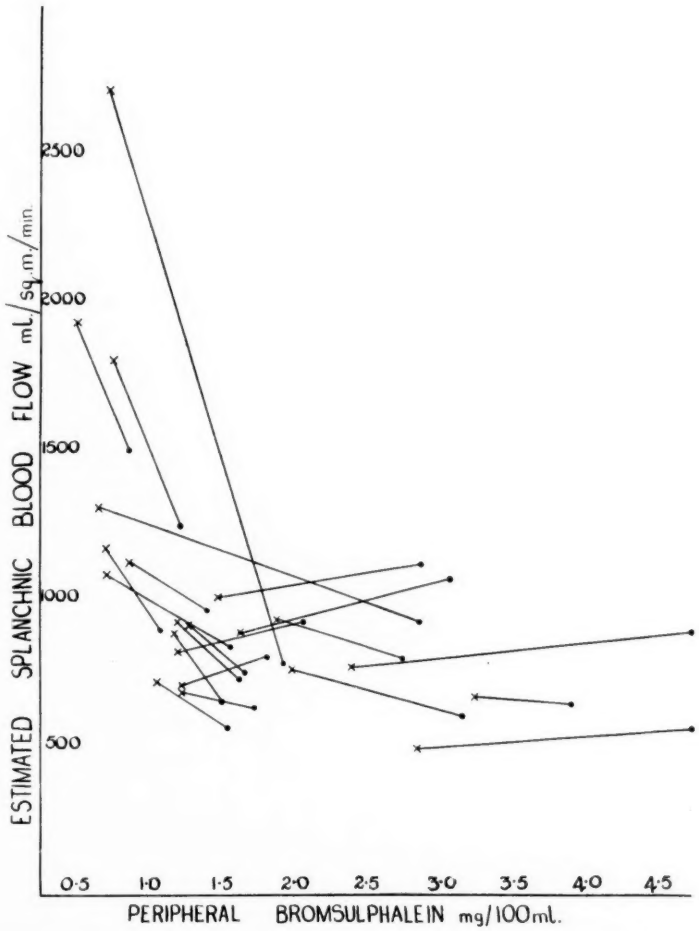

Fig. 2.—Changes in calculated splanchnic blood flow related to alteration in peripheral venous bromsulfalein. × represents initial reading and • the value obtained 2 to 3 hours later.

Hepatic Bromsulfalein Extraction.—The percentage extraction of BSP was greater at the lower peripheral BSP levels. The peripheral level was plotted on semilog paper against percentage extraction (Fig. 4). The values obtained are comparable with those given by Myers[9] for his control series and provide evidence that hepatocellular function with respect to BSP was normal in the present group.

Constancy of the Estimated Splanchnic Blood Flow Over Long Periods.—In fifteen subjects the initial peripheral BSP value was greater than 1 mg. per 100 ml. and the calculated splanchnic blood flow was compared with that obtained two hours later (Fig. 2). E.S.B.F. showed little change.

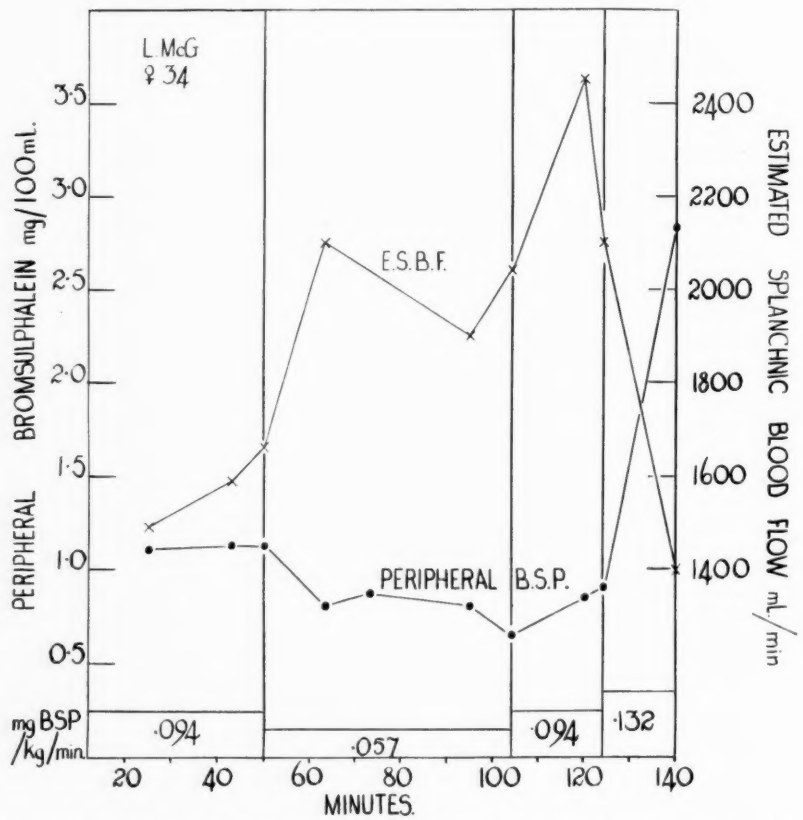

Fig. 3.—Effect of changing dose of bromsulfalein on peripheral bromsulfalein values and calculated blood flow.

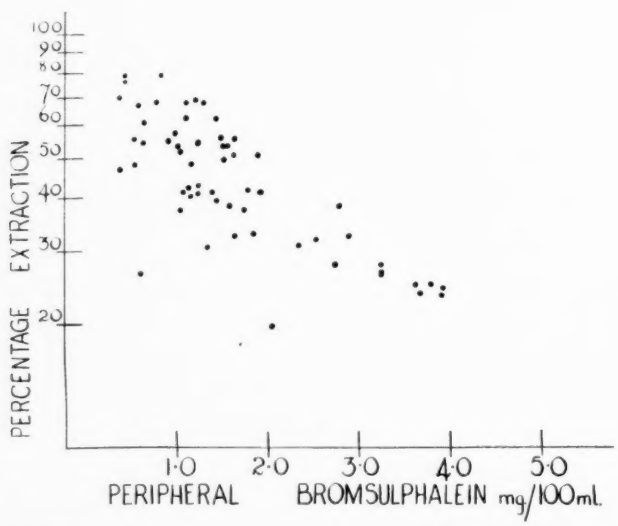

Fig. 4.—The relation of peripheral bromsulfalein values to the percentage extraction by the liver.

TABLE III. REPEATED ESTIMATIONS OF SPLANCHNIC BLOOD FLOW IN TWO NORMAL SUBJECTS

SUBJECT	AGE	SEX	WT. (KG.)	S.A. (SQ.M.)	BSP		E.S.B.F. (ML./MIN.)								
					INITIAL PERIPHERAL VENOUS VALUE (MG./100 ML.)	DOSE (MG./KG./MIN.)	TIME IN MINUTES FROM START OF BSP INFUSION								
							35	45	60	75	90	110	130	150	170
M. H.	60	M	49	1.56	2.07	0.112	1250	1050	1030	—	1200	1070	1000	990	—
A. B.	40	M	64	1.74	1.15	0.072	—	1600	—	1510	1530	1380	1350	1650	1330

In two normal subjects more detailed studies were made. The results are tabulated in Table III. It is seen that, with the catheter remaining in the same branch of the hepatic vein, range of variability in E.S.B.F. is of the order of 300 ml. per minute.

DISCUSSION

The very high calculated splanchnic blood flow, encountered when the peripheral BSP value was low, was an unexpected finding. Bradley and co-workers,[2] using a similar technique, did not describe this difficulty, even though in thirteen of their twenty-three observations the BSP level was less than 1 mg. per 100 milliliters. It must be emphasized that both the high and low peripheral BSP values reported in the present work resulted from BSP infusion, in amounts comparable to those used by Bradley and associates.

Our anomalous results for E.S.B.F. can be related to the mechanisms for the removal of BSP from the blood. Although the liver may be the principal organ involved,[2, 10, 11, 12] there is other evidence that extrahepatic sites and, in particular, the reticuloendothelial system play an important part in its disposal.[13-17] If BSP is removed from the blood by sites other than the liver, the quantity infused would not correspond with that excreted by the liver. The numerator in the equation used to calculate E.S.B.F. would thus be too high and falsely high values for splanchnic blood flow would be obtained. This might explain the incredibly high values for E.S.B.F. found when the peripheral BSP level is low.

Wirts and Cantarow[15] have shown that 67 to 100 per cent of injected BSP is recoverable in the bile within two hours, and although the reticuloendothelial system may participate initially in the withdrawal of the substance from the blood stream, the liver is ultimately responsible for its removal from the body. When the peripheral BSP level is increased, then the extrahepatic sites of removal may become saturated with the dye. The rate of entry of BSP into these sites then equals the rate of removal and the liver receives and excretes the exact quantity that is being infused. The Fick principle becomes applicable and correct values for E.S.B.F. are obtained.

Provided the peripheral BSP level is greater than about 1 mg. per 100 ml., raising the level further will not produce a further depression of the calculated splanchnic blood flow. Saturation of any extrahepatic removal mechanisms appears to be complete. This is in contrast to the findings of Cohn and associates[18] in the eviscerated-hepatectomized-nephrectomized dog. With this preparation these authors could demonstrate removal of BSP from the blood by other tissues in increasing amounts, even with plasma concentrations as high as 100 mg. per 100 milliliters. It is impossible, however, to compare the results obtained from this preparation with those obtained from man where the liver must be playing the major role in BSP removal.

Mendeloff and co-workers[19] refer to saturation of the liver cells with BSP at high peripheral concentrations, particularly in patients with liver disease. They believe that it is under these circumstances that extrahepatic removal of the dye may occur. The present work shows that increasing the peripheral

BSP level from 1 to 4 mg. per 100 ml. does not lead to any change in E.S.B.F. (Fig. 2). Extrahepatic withdrawal of the dye is therefore unlikely to be a serious source of error at these levels.

Anomalously high calculated splanchnic blood flows are obtained whether the low peripheral BSP value occurs initially or follows a previous high value (Fig. 3). Saturation, therefore, is probably controlled by the peripheral BSP level.

Reliable estimates of E.S.B.F. by this technique will not be obtained unless the peripheral venous BSP is greater than 1 mg. per 100 milliliters. The first venous blood sample obtained should, therefore, be analyzed immediately; if the BSP concentration obtained is not adequate, then the concentration of the infusion fluid must be increased. Prediction of the strength of infusion required in any one subject cannot be assessed entirely on the age and size of the subject and the preliminary estimation is essential. The rate of increase should be small so that the necessary correction can be made. Under these conditions, the liver and extrahepatic sites of removal are working at maximal capacity in the disposal of BSP from the blood stream. The conditions are similar to those employed in measuring the maximal tubular capacity (Tm) of the kidney. All measurements of E.S.B.F. by this technique therefore necessarily measure maximal hepatic capacity for BSP (Lm).[20]

Provided these conditions are satisfied, the BSP extraction technique has proved a satisfactory method of measuring splanchnic blood flow for periods of as much as three hours.

SUMMARY

The estimated splanchnic blood flow (E.S.B.F.) has been determined in forty-nine normal subjects by the bromsulfalein (BSP) extraction technique using hepatic vein catheterization.

In thirty-two subjects, the peripheral venous BSP level was greater than 1 mg per 100 ml. and the E.S.B.F. was 836 ± 31 ml. per minute per square meter. In twelve subjects the peripheral BSP value was less than 1 mg. per 100 ml. and the E.S.B.F. was 1530 ± 126 ml. per minute per square meter.

When the peripheral BSP level was less than 1 mg. per 100 ml., significant diminution in the calculated splanchnic blood flow could be induced by raising the peripheral concentration. Increases to values greater than 1 mg. per 100 ml. failed to produce any further significant change in E.S.B.F. The reverse finding, that reduction of peripheral BSP values from above 1 mg. per 100 ml. to below that value increased the apparent E.S.B.F., was also true.

These results suggest that, unless extrahepatic removal mechanisms for BSP are saturated, values for E.S.B.F. are falsely high. With adequate peripheral BSP levels, changes in E.S.B.F. have been followed for periods of three hours and have shown only small fluctuations.

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THE EFFECT OF RUTIN ON CAPILLARY FRAGILITY AND PERMEABILITY

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THERE is currently much interest in the vascular disease associated with hypertension and with diabetes mellitus. Hemorrhagic complications are assumed by many to be due to increased capillary fragility.

In 1936, Szent-Gyorgyi and co-workers[1] isolated from lemons a compound which they called citrin, subsequently found to consist of three flavone derivatives—hesperidin, eriodictyol glucoside, and a quercitrin-like substance.[2] They believed it to be helpful in purpura where vitamin C was not beneficial and therefore named it vitamin P (permeabilitäts-vitamin).

Gyorgyi and Rusznyak[3] suggested that ascorbic acid is accompanied in the cell by vitamin P and that experimental scurvy is caused by the continued lack of both vitamins C and P, for guinea pigs on a Sherman-LaMer-Campbel scurvy diet lived an average of 15.5 days longer than controls when given citrin.[4] Pure P avitaminosis produced no clinical symptoms, while the pathology of C deficiency was greatly modified when P was also withheld. The flavone hesperidin was inactivated when converted to the corresponding flavonol.

The flame of enthusiasm for citrin was practically extinguished when Gyorgyi[5] reported his failure to repeat his original experiments and with the failure of Moll[6] and Zilva[7] to find any basis for the existence of a vitamin P.

Before long, enthusiasm was rekindled by Scarborough and Stewart's[8] report that oral administration of hesperidin reduced the number of hemorrhages in patients with vitamin deficiency independent of the presence of ascorbic acid in the diet. Jersild[9] suggested that Schönlein-Henoch purpura is caused by deficiency of vitamin P. Scarborough[10] next described two different forms of subcutaneous bleeding, one due to a deficiency of ascorbic acid and the other resulting from a deficiency of vitamin P.

As late as 1944 the controversy continued. Rudy, Beaser, and Seligman[11] reviewed the literature wherein citrin has been tried in a wide variety of cases with increased capillary fragility irrespective of the underlyng disease and criticized the conclusions which they believed to be invalid because of unreliable test methods. During the same year, Griffith and Lindauer[12] reported that hesperidin and hesperidin methyl chalcone restored capillary fragility to normal in about 84 per cent of cases of hypertension showing increased fragility.

Subsequently, a new era of confusion was at hand. Couch, of the Eastern Regional Research Laboratories of the United States Department of Agriculture, noted the chemical similarities between rutin, a flavonol glucoside found in many plants, and hesperidin. He thought it possible that rutin might have a beneficial effect on abnormally fragile capillaries, although Gyorgyi had previously stated that the flavonol derivatives were inactive. Rutin was first given to patients with hypertension who showed an increased capillary fragility by Griffith and co-workers[13]; the results were favorable and it was concluded that rutin has an effect similar to hesperidin. Shannro[14] concluded that rutin was possibly superior to hesperidin. It seemed reasonable to him that the drug might prevent vascular accidents in patients with hypertension, maintaining normal capillary fragility and even controlling pulmonary bleeding when no other cause was apparent. Johnson[15] boldly recommended its use in hypertension and diabetic retinitis, mentioned its nutritional value, and expressed the opinion that it may serve the circulatory system in a manner similar to that of vitamin C in the growth and hardness of teeth and bone. He thought 1,300,000 pounds

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would be required annually. Freeman[16] suggested the synchronous use of rutin with thiouracil because of the questionable deleterious effect of the latter drug on blood vessels. Kushlan[17] thought rutin therapy might represent the long sought for specific remedy for hereditary hemorrhagic telangiectasia. Griffith[18] concluded that rutin appeared to hasten the recovery time after irradiation injury in rats. Zfass[19] concluded that rutin might prove to be a valuable drug in preventing cerebral vascular accidents and retinal hemorrhages occurring in hypertension.

The Council on Pharmacy and Chemistry[20] warned (1946) that few data had been presented on variability of capillary fragility in untreated cases of hypertension, that increased capillary fragility is not a common complication of hypertension, and that there was no agreement among workers in this field on the reliability of any of the methods of measuring capillary resistance. They recapitulated the experience with hesperidin or vitamin P and expressed the hope that history would not repeat itself in the case of rutin.

Wolffe and Danish[21] reported the occurrence of subconjunctival hemorrhages in patients receiving rutin; they speculated that there is a "vulnerable period" early in the administration of rutin during which an increased tendency to hemorrhage exists. Soloff and Bello[22] could not demonstrate any beneficial effects from the administration of rutin in hypertensive subjects who had previously been treated with vitamin C. Rodriquez and Root[23] followed twenty diabetic patients who were receiving rutin and found that the capillary fragility became normal or borderline in 25 per cent, remained positive but improved in 25 per cent, and was not improved in 50 per cent. They noted no improvement in the retinal picture or visual fields of these patients although several were strongly convinced that rutin had improved their vision. Beardwood and co-workers[24] studied 116 diabetic subjects with retinopathy divided into forty receiving rutin, forty receiving hesperidin, and thirty-six receiving placebo. Of the eighty patients receiving rutin or hesperidin, 25 per cent showed definite improvement on ophthalmologic examination. Their data revealed a far greater favorable effect on the Göthlin index within the groups receiving rutin and hesperidin than within the group receiving the placebo. They suggested the use of rutin for prophylaxis against intercapillary glomerulosclerosis. Hollenhorst[25] and Sokoloff and Redd[2] have reviewed the literature in which rutin had been given a clinical trial and concluded that further study was desirable.

The use of rutin for the treatment or control of hemorrhagic phenomena in diabetes and hypertension is predicated on the assumption that in these diseases the capillaries are abnormally fragile or permeable. Whether fragility and permeability are related is not apparent. Since the movement of fluid from a capillary takes place at least chiefly through the intercellular cement spaces,[26] and since rupture of a capillary would seem more likely to occur at an intercellular face than across an endothelial cell, it seems reasonable to suspect that a relation between the two may exist. The common association of retinal exudate and hemorrhage is consistent with such a hypothesis, as well as the petechiae of scurvy and the belief that ascorbic acid is effective by its action on "cement substance."

Unfortunately, no satisfactory methods are available for the clinical estimation of either capillary fragility or permeability. Attempts have been made to estimate capillary fragility by one of two methods, negative or positive pressure. In the former, the minimum negative pressure which, when applied to a cutaneous area for a given time, produces visible petechiae is recorded. In the latter, capillary pressure is increased by a tourniquet and the appearance of petechiae after a specified time is recorded. Numerous techniques for each method have been used.

The negative test was introduced by Hecht[27] in 1907 and modified by Borbely,[28] Dalldorf,[29] and others. Rudy[30] believed it to be unreliable because contiguous areas of one arm showed marked variations. For the positive pressure or tourniquet test, both supradiastolic and infradiastolic pressures have been used. Rumpel,[31] Leedes,[32] and Wright[33] used supradiastolic pressures. In hypertensive patients, Rudy believed that the pressure at which the test was performed was responsible for the high percentage of positive reactions rather than the hypertension itself.

Göthlin[34] counted the number of petechiae appearing in a 6 cm. circle after pressures of 35 and 50 mm. Hg and proposed a petechial index, which, with various modifications, has been commonly used.

Because of the variability in the appearance and disappearance of petechiae in hypertensive and diabetic patients, it was felt that any study of the possible effects of rutin should include control groups of cases as nearly comparable as possible. Also, unless an effect on capillary fragility or permeability could be demonstratel by available methods, admittedly imperfect as these are, any confidence in the value of rutin would have to be based on a long follow-up of an adequate number of treated and control cases.

MATERIALS AND METHODS

The plan of this study was to follow patients with diabetes and with hypertension, who showed petechiae or retinitis, by periodic estimation of capillary fragility, and of capillary permeability by the Landis test,[35] giving alternate subjects rutin and a placebo. Thirty-one patients, sixteen treated and fifeen control, were followed for one to three months without even a suggestion that rutin in the dosage used (180 mg. daily) had any demonstrable effect. Three patients with Laënnec's cirrhosis and many petechiae, given rutin, likewise showed no change. It therefore did not seem profitable to continue this type of study. Since this negative result is at variance with the majority of reports in the literature, it seems pertinent to report the technique used.

Diabetic and hypertensive patients attending the Out-patient Clinic who showed retinal hemorrhages or exudate or petechiae were selected. A medical history, with special emphasis on diet and hemorrhagic phenomena, was obtained; physical examination with careful observation of the retinas was carried out; and negative pressure and Göthlin tests were performed at the first session. Approximately one week later the Landis test was carried out, and the patient was given either rutin* or placebo. Tests were repeated in the same manner at approximately monthly intervals.

The negative pressure test was carried out according to Dalldorf's[29] technique, using a capsule 1 cm. in diameter with a flat 8 mm. rim. Pressure was applied for one minute to areas on the outer and inner aspects of the antecubital fossa and the volar surface of the wrist of each arm. The recorded figure is the average of the minimum negative pressure required to produce petechiae in each area. A capillary resistance of 200 mm. Hg or less was considered abnormal.

For the Göthlin index, circular areas 6 cm. in diameter were marked on each antecubital fossa, and spots which might be confused with petechiae were marked with ink. With the patient recumbent and the arms elevated to heart level on sandbags, blood pressure cuffs were applied to each arm and one quickly was inflated to 35 mm. Hg and the other to 50 mm. Hg from a compressed air line, regulated by screw clamps and a side arm with a constant leak. Pressure was maintained for fifteen minutes. The patient held his arms elevated for five minutes, after which the number of petechiae in each circle was counted by

*Generous supplies of rutin were furnished by Dr. L. E. Josselyn of the Abbott Research Laboratories.

TABLE I. MEASUREMENTS OF CAPILLARY FRAGILITY AND PERMEABILITY IN PATIENTS RECEIVING RUTIN AND IN CONTROLS

SUBJECT	SEX AND AGE	DATE	B.P.	CAPILLARY FRAGILITY		CAPILLARY PERMEABILITY		RETINAS		PETECHIAE
				NEG. PRES. (MM. HG)	GÖTHLIN INDEX	FLUID LOSS (C.C./100 C.C.)	PROTEIN (% in EDEMA)	HEMORRHAGE	EXUDATE	
NORMAL CONTROLS										
R. L.	M 30	7/48	130/80	375	0	7.3	1.4	0	0	0
W. M.	M 24	8/48	130/85	290	2	8.7	1.1	0	0	0
R. F.	M 24	8/48	110/70	315	0	9.4	0.3	0	0	0
G. M.	M 24	10/48	120/50	250	8	6.2	0.3	0	0	0
R. R.	M 27	10/48	120/76	250	0	6.8	1.2	0	0	0
DIABETIC CONTROLS										
R. C.	F 60	9/48	182/86	225	0	4.7	1.1	+	+	0
		10/48	185/85	250	10	7.8	1.8	++	++	0
		11/48	190/94	210	26	2.1	2.4	+	+	++
E. N.	F 64	9/48	210/77	340	2	2.7	4.8	0	+	0
		10/48	187/70	375	0	4.9	3.9	0	+	0
		11/48	187/70	290	2	6.2	1.9	0	+	++
H. W.	M 17	9/48	122/80	560	0	7.6	2.8	+	0	0
		10/48	118/65	560	4	6.5	2.3	+	0	0
		12/48	120/65	450	0	3.1	0.0	+	0	0
A. H.	F 66	12/48	155/80	90	54	7.9	0.6	+	+	+
		2/49	130/70	115	36	8.0	2.0	+	+	+
		3/49	130/60	85	54	7.8	1.4	+	+	++
S. M.	F 33	9/48	190/105	210	6	5.9	1.0	+	+	+
		11/48	190/105	190	8	5.3	1.9	+	+	+
		12/48	190/105	225	10	4.9	1.2	+	+	+
H. P.	F 67	11/49	190/88	100	14	4.0	7.4	+	+	+
		12/49	188/78	75	8	0.9	1.0	+	+	+
		12/49	186/76	105	15	4.7	6.4	+	+	+
B. B.	F 64	10/49	128/70	155	6	2.0	0	+	++	+
		11/49	145/70	95	16	1.5	0	+	++	-
		12/49	130/70	130	24	1.6	2.5	+	++	0
H. R.	M 67	10/49	140/90	145	0	- 0.5	0	+	+	0
		11/49	136/78	120	0	- 1.0	0	+	+	0
		12/49	140/68	155	0	- 1.3	0	+	+	0
H. A.	M 66	10/49	150/90	135	2	1.0	0	+	0	+
		11/49	142/80	95	2	2.2	2.7	+	0	-
		12/49	140/74	150	4	1.9	3.2	+	0	0
DIABETICS RECEIVING RUTIN										
F. W.	M 64	8/48	165/75	175	10	8.9	1.1	0	+	+
		10/48	165/80	190	40	10.2	0.1	+	+	++
		11/48	155/85	190	30	7.7	1.3	+	+	+
I. R.	F 66	9/48	205/70	250	48	8.5	2.5	+	+	-
		10/48	195/60	285	38	11.2	3.4	+	+	-
		11/48	190/70	265	24	3.3	0.3	+	+	+
J. F.	M 68	9/48	160/80	140	52	7.2	3.1	+	+	+
		10/48	150/85	125	18	5.1	0.4	+	+	+
		12/48	155/80	125	20	7.5	0.0	+	+	+
M. C.	M 63	9/48	130/65	110	16	12.9	0.0	+	+	0
		11/48	120/65	115	6	7.8	1.5	+	+	+
		12/48	130/65	100	10	18.0	1.9	+	+	+

(*Continued on opposite page.*)

TABLE I—CONT'D

SUBJECT	SEX AND AGE	DATE	B.P.	CAPILLARY FRAGILITY		CAPILLARY PERMEABILITY		RETINAS		
				NEG. PRES. (MM. HG)	GÖTHLIN INDEX	FLUID LOSS (C.C./100 C.C.)	PROTEIN (% in EDEMA)	HEMORRHAGE	EXUDATE	PETECHIAE
L. O.	F	12/48	185/85	75	36	8.5	0.2	+	++	++
	60	1/49	185/85	60	28	7.6	0.0	+	++	++
		2/49	185/85	65	42	11.3	1.6	+	++	+
L. H.	F	10/49	184/120	300	2	5.9	–	0	+	0
	67	11/49	180/120	360	0	0.7	5.7	0	+	0
		12/49	160/116	325	0	5.9	5.3	0	+	0
C. R.	F	10/49	194/108	255	0	4.1	0.7	++	+	+
	67	11/49	214/100	185	2	9.1	1.4	++	+	+
M. T.	F	10/49	170/96	230	4	4.2	3.5	0	+	0
	66	11/49	180/90	390	0	3.0	2.6	+	+	0
		12/49	160/90	510	0	0.5	(–2.0)	+	+	0
A. V.	F	10/49	198/88	205	2	–	–	+	+	6
	60	11/49	180/80	130	10	4.6	7.4	+	+	0
		12/49	186/86	130	4	2.9	–	+	+	+
H. W.	F	11/49	140/90	285	2	1.1	7.0	+	+	0
	46	12/49	170/110	355	0	–	–	+	+	0
		12/49	180/110	325	2	–	–	+	+	0
HYPERTENSIVE CONTROLS										
F. P.	M	9/48	200/115	240	36	9.0	0.0	+	++	+
	71	10/48	190/115	200	18	10.6	0.7	+	++	++
		11/48	200/115	200	28	2.6	0.0	+	++	++
A. M.	M	9/48	210/140	590	0	7.8	3.8	+	+	0
	60	10/48	195/115	580	2	2.1	0.0	0	+	0
		11/48	205/125	600	0	4.7	0.6	0	+	0
W. P.	M	10/48	175/130	300	2	9.6	2.1	0	+	+
	49	11/48	155/90	275	4	2.7	0.0	0	+	+
		1/49	170/105	250	16	8.7	0.5	0	+	+
A. W.	M	8/48	195/135	540	2	3.5	5.1	+	0	+
	32	10/48	195/140	600	8	5.4	2.4	+	0	+
A. Y.	F	11/49	202/120	166	4	8.8	5.9	+	+	0
	68	12/49	200/110	165	0	3.3	5.8	+	+	0
		12/49	185/110	150	0	3.1	0.0	+	+	0
K. D.	F	11/49	196/96	165	14	0.6	0.0	+	+	0
	66	12/49	190/100	160	2	1.2	1.7	+	+	0
HYPERTENSIVES RECEIVING RUTIN										
M. V.	F	8/48	220/125	125	14	5.1	4.7	+	+	0
	43	9/48	200/125	135	14	9.2	0.0	+	+	0
		10/48	220/120	135	28	7.1	1.4	+	+	0
L. L.	F	12/48	250/95	185	14	7.4	1.8	+	+	++
	70	1/49	270/95	160	18	7.0	1.3	+	+	+
		2/49	250/90	150	16	12.7	1.6	+	+	++
W. C.	M	1/49	230/115	140	8	8.1	1.6	+	+	+
	61	2/49	240/115	135	10	8.9	1.2	++	+	++
		4/49	240/115	175	36	12.0	1.9	+	+	+
J. H.	M	9/49	235/150	290	14	6.1	1.0	+	+	+
	29	10/49	195/136	350	66	–	–	+	+	+++
R. L.	F	10/49	212/130	210	0	12.1	0.3	+	+	0
	43	11/49	208/120	310	4	6.3	2.4	+	+	0
		12/49	212/126	290	0	8.9	1.5	+	+	0
E. H.	F	10/49	200/110	255	0	9.7	6.0	+	0	0
	66	12/49	225/130	305	0	2.0	0.0	+	0	0
		1/50	202/120	405	0	6.6	3.9	+	0	0

strong artificial light, using a magnifying lens and a circular plane glass for locally pressing the skin to distinguish between hyperemic spots and petechiae. In order to compare these results with the two-stage technique used by Griffith and others, the number of petechiae found in the 6 cm. circle at 35 mm. Hg was multiplied by four and added to the number in the 50 mm. Hg circle multiplied by two to give the "index." An index of twelve or more was considered abnormal.

RESULTS

Landis' test is an attempt to measure capillary permeability by estimating the amount of fluid that leaves the blood vessels and its protein content when venous pressure is raised to 40, 60, or 80 mm. Hg. The method has been severely criticized by Bing,[36] who found it totally unreliable when performed at 40 mm. Hg pressure. Landis found much more consistent results at the higher pressures. We used a pressure of 9 and 60 mm. Hg, observing the precautions outlined by Landis and the same analytical methods. A fluid loss of 12 c.c. per 100 c.c. blood or a calculated protein concentration of more than 2 per cent in the edema fluid was considered abnormally high. The retinas were examined at each visit for the presence of hemorrhages and exudate, and the skin was inspected for petechiae. These were recorded qualitatively as one to four plus. Table I summarizes the pertinent data from all groups of patients studied.

According to the criteria used, five of nine diabetic controls and four of ten treated diabetic subjects showed an increased capillary fragility to negative pressure when first seen which remained unchanged except for one treated diabetic subject who showed improvement. The Göthlin index remained positive in two of the control group and became positive in two more, while in those receiving rutin three stayed positive, one became negative, and one developed a positive test while under treatment. No consistent abnormality in capillary permeability, or change under treatment, was detected by the Landis test. There was no significant change in the appearance of the retinas in either group while under observation. Three controls and three treated patients developed more petechiae, while one of the latter showed improvement. Observations on the patients with hypertension were similarly undramatic.

CONCLUSION

No change in capillary fragility as measured by positive and negative pressure methods, in capillary permeability, in retinal hemorrhages and exudate, or in petechiae was detected in ten patients with diabetes mellitus and six hypertensive subjects given 180 mg. rutin daily for one to three months when compared with similar patients given a placebo.

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STUDIES ON BETA-GLUCURONIDASE ACTIVITY IN PREGNANT ALBINO RATS

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ONE of the fundamental conjugating mechanisms in the body involves the joining together of glucuronic acid with toxic substances containing a free hydroxyl group. Among the many substances reported as participating in this reaction are menthol,[1] estriol,[2] and diethylstilbestrol.[3] Products of the reaction are hydrolyzed in vitro by an enzyme in animal tissues, beta-glucuronidase.

The activity of the enzyme, beta-glucuronidase, in human beings has been investigated in respect to normal and abnormal pregnancy[4] and menstruation[5] and to cancer.[6] It was observed that the activity in serum increased fourfold during normal pregnancy and was still higher during toxemic pregnancy. Moreover, changes in activity of homogenized endometrium were observed during the normal menstrual cycle, more activity being observed during the late proliferative phase of endometrial development than during the resting and late secretory phases. Recently, increased activity was found in malignant tissues of the cervix, vagina, and vulva when compared with nonmalignant tissues of the same organ.

In animals, the activity of beta-glucuronidase has been reported after the administration of borneol, menthol, and steroids.[7, 8] With the first two compounds increases in glucuronidase activity were noted in liver, spleen, and kidney but not in the uterus. On the other hand, an increase in the uterine activity of castrates was found after administration of estrogens, but there was no change in the enzyme in the kidney, spleen, and liver. To explain these results it was suggested that glucuronidase can act synthetically and that glucuronides of the terpenes are formed in the liver, spleen, and kidney, while those of the steroids are formed in the uterus. Kerr and Levvy,[9] however, found an increase in the enzyme activity of liver as well as uterus in ovariectomized mice following the administration of estrone. Moreover, the increase in enzyme activity in both organs following the administration of compounds such as carbon tetrachloride and chloroform in toxic doses can be explained more readily on the basis of a relation to cellular proliferation rather than to glucuronide synthesis.*

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*The effect of estrogens (biologic assay) on glucuronidase activity in uteri from immature white mice was studied by Scott (personal communication). Whereas uterine weight was observed to increase following the administration of fluids containing estrogen, the glucuronidase activity did not materially change per gram of tissue assayed. The dry weight (total water) of the uterus was not determined.

It is noteworthy that no assays for glucuronidase activity have been made on the experimental animal during pregnancy. In view of the increase during gestation in the blood and urine levels of steroids, some of which are conjugated with glucuronic acid, such a study would offer evidence concerning the interrelated effects of these endogenous glucuronidogenic substances and the enzyme in the liver, spleen, kidney, and uterus.

MATERIAL AND METHOD

Homozygous mature white rats, weighing between 225 and 250 grams, were mated with males from the same strain. Day one of pregnancy was established as the day when sperm were first found in the vagina. Groups of five rats were sacrificed seven, fourteen, eighteen, and twenty days thereafter and one and seven days post partum. Six nonpregnant female rats served as controls. Blood was collected by intracardiac puncture subsequent to decapitation. Commencing with 14 days of gestation the uterus was incised in an area distal from the attached placenta, so that the embryo, placenta, uterine wall, and placental site could be assayed separately. Wet tissues weighing 50 to 100 milligrams were homogenized with water. Assays on blood and tissues were made according to a modification[10] of the method described by Talaly and co-workers.[11] The method employs phenolphthalein glucuronide as substrate. Assays were made in triplicate. Since rich sources of glucuronidase activity require short periods of incubation, a water bath at a temperature of 38° C. was used instead of an air incubator. Units of activity are expressed as the gammas of phenolphthalein liberated per gram of tissue or per 100 ml. of fluid per hour of incubation.

RESULTS

The results with their statistical interpretations are given in Tables I and II. Definite changes were evident. These may be stated as follows. The glucuronidase activity in the liver and blood increased during pregnancy. In the former, the increase continued during the first seven days post partum, whereas in the latter, enzyme activity decreased after delivery. A decrease was observed in the activity in the ovary during pregnancy. The changes in the activity in the uterus deserve special comment. The placental site showed an increase in activity of enzyme, whereas uterine wall from other areas remained low in activity during the period of gestation when compared with uteri of nonpregnant control animals. It is of interest that the highest activity observed

TABLE I. GLUCURONIDASE ACTIVITY

(FIGURES* EXPRESS γ PHENOLPHTHALEIN LIBERATED PER GRAM OF TISSUE PER HOUR OF INCUBATION)

DAYS OF GESTATION	NUMBER OF ANIMALS	LIVER	UTERUS†		OVARIES	SPLEEN
			D	P		
Control	6	14,000 ± 2,000	6,300 ± 1,250		13,600 ± 1,430	20,600 ± 3,810
7	5	18,650 ± 1,770		2,580 ± 505‡	12,950 ± 2,670	24,500 ± 7,360
14	5	28,200 ± 7,120	4,000 ± 1,630	20,700 ± 4,050	7,760 ± 1,920	23,700 ± 3,780
18	5	25,400 ± 3,740	2,690 ± 175	24,100 ± 2,860	8,310 ± 1,258	22,000 ± 2,130
21	5	25,300 ± 8,980	2,070 ± 482	29,700 ± 7,150	7,020 ± 1,340	23,800 ± 3,460
1 day p.p.	5	27,200 ± 7,200	3,880 ± 2,000	26,200 ± 8,490	10,400 ± 1,930	28,250 ± 6,710
7 days p.p.	5	35,150 ± 3,050		20,000 ± 6,920	16,220 ± 3,250	30,900 ± 5,830

*Mean and standard deviation.

†Uterus: D, tissue from uterus not containing placental site; P, tissue from uterus containing placental site.

‡Tissue assayed contained uterus with intact gestational products. The activity in fetal liver was an average of 2,830 units.

in the rat (60,000)* occurred in tissues containing the placental site. The activity in kidney, vagina, jejunum, and lungs was not remarkably affected during pregnancy. This was also true of the thyroid and brain. The activity in the homogenized fetus and in the amniotic fluid decreased toward the end of gestation. In addition, fetal liver was found to be lower in activity than the maternal organ. Activity in the spleen, although not affected significantly by pregnancy, tended to increase during gestation and during the seven days post partum; in addition the spleen showed greater activity on the average than other organs except uterus (placental site) and liver.

TABLE II. GLUCURONIDASE ACTIVITY
(FIGURES* EXPRESS γ PHENOLPHTHALEIN LIBERATED PER GRAM OF TISSUE OR 100 ML. FLUID PER HOUR OF INCUBATION)

DAYS OF GESTATION	NUMBER OF ANIMALS	PLACENTA	FETUS	AMNIOTIC FLUID	BLOOD SERUM
Control	6				206 ± 104
7	5				594 ± 153
14	5	3,550 ± 1,290	1,500 ± 500	10,500 ± 4,950	417 ± 189
18	5	4,910 ± 890	1,090 ± 260	3,610 ± 2,700	468 ± 50
21	5	4,700 ± 799	530 ± 59	2,360 ± 570	883 ± 410
1 day p.p.	5				474 ± 222
7 days p.p.	5				470 ± 185

Other tissues assayed but not changing significantly in activity during pregnancy varied as follows: kidney, 3,140 to 4,310; vagina, 1,920 to 6,000; thyroid, 2,010 to 2,710; thymus, 4,300 to 7,550; adrenals, 6,335 to 9,290; jejunum, 4,950 to 5,530; lungs, 3,470 to 4,070; corpus luteum, 9,780; breast, 3,500 to 8,100; brain, 35 to 357; muscle, 101 to 180; heart, 220 to 479.

*Mean and standard deviation.

DISCUSSION

Despite a variation in results, the changes occurring in the serum, liver, ovaries, uterus, fetus, and amniotic fluid are considered significant. It is of interest that the increased activity of blood serum is similar to that observed during human pregnancy.[4] The decrease in ovarian enzyme occurs during a period of cellular inactivity in that organ. In addition, the decrease in activity in the homogenized fetus as the gestation progresses suggests that the more rapidly growing tissues of the younger embryo are richer sources of enzyme, an observation which agrees with a report by Kerr and Levvy.[9] The decrease in activity in amniotic fluid observed during the later phase of gestation may be a dilution phenomenon from secretion by the fetal kidneys. It has been observed, for example, in human beings that the urine of newborn infants is unusually low in glucuronidase activity.[12]

It has been reported that the liver inactivates circulating estrogens or at least changes them to a less active form.[12] Since estriol glucuronide is biologically less potent than free estriol, some of the "inactivation" could possibly involve a glucuronide synthesis in which it has been suggested beta-glucuronidase could participate.[7] In which case it would seem that the conjugating ability of the maternal liver, in contrast to the fetal liver, is increased during pregnancy. On the other hand, changes in activity of beta-glucuronidase in tissues have been reported as being unrelated to the tissues' ability

*A single determination.

to synthesize glucuronides in vitro. Karunairatnam and co-workers[14] found that during the period of enhanced glucuronidase activity in liver following partial hepatectomy, and the administration of carbon tetrachloride or menthol, the ability to synthesize glucuronides remained normal. Also it was unchanged during the depression of glucuronidase activity which follows sorbic acid administration; and tumors with increased glucuronidase activity were devoid of power to synthesize glucuronides. In this respect it is of interest that Lipschitz and Bueding[15] found the ability to synthesize glucuronides largely confined to the liver, although other organs such as spleen, kidney, and uterus have unusually enhanced glucuronidase activity. Fishman's theory,[7] that beta-glucuronidase activity is increased in tissues following the administration of glucuronidogenic substances as an adaptation for metabolic conjugation, is not supported by Levvy and associates.[16] They observed an increase in activity of enzyme in the liver during a period of cellular regeneration following the administration of nonglucuronidogenic compounds, such as chloroform and phosphorus. Thus, the reason for the increasing activity in the rat uterus during pregnancy reported in this study is not certain. Whether it portends an unusual glucuronide synthesis of steroids by that tissue (Fishman) or is merely due to the cellular activity (mitosis) occurring in the placental site (Levvy) cannot be determined on the basis of present knowledge. Furthermore, which of the cells of the placental site contain the enzyme is unknown. Histologic examination of the placenta and underlying tissues removed during pregnancy would seem to implicate adherent trophoblastic cells; further studies are being carried out in this direction.

Some mention should be made regarding the comparison of values obtained on rat tissues with those reported on human tissues. A similar wide range of activity was found in the human tissues studied. The normal human endometrium[5] was observed to vary in activity from 895 to 9,040 units; and this range was found to be greater in specimens of endometrium removed from women with functional uterine bleeding.[17] The uteri (rat) reported in the study (Table I) were removed at approximately the same stage of endometrial development (Stage II or III), which may account for less variation in results. The activity in human decidua[17] was found to be highest in early pregnancy (under twelve weeks of gestation), attaining in some cases 23,000 units. Excepting placental site, the activity in uterine wall containing decidua declined below 1,000 units after twelve weeks of human gestation. With the exception of some isolated values[4] obtained from post-mortem specimens, no satisfactory figures are available on the activity of enzyme in other organs during human pregnancy.

SUMMARY

The activity of the enzyme, beta-glucuronidase, was determined in tissues and blood of female albino rats during pregnancy. An increase in the activity in serum, liver, and uterus was observed during gestation. A decrease occurred in ovary, homogenized fetus, and amniotic fluid. No changes of statistical significance were noted in spleen and kidney, or in the various other tissues assayed.

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VENTILATION MEASUREMENTS IN PULMONARY EMPHYSEMA TREATED WITH PNEUMOPERITONEUM

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CHRONIC pulmonary emphysema has defied attempts at definite therapy in spite of numerous thorough investigations concerning its physiopathology. Although its origin is probably intrapulmonary, the late stages of the disease are characterized by impaired thoracic and diaphragmatic function as well as damaged lung parenchyma.

Previous therapy has been directed toward restoration of functional efficiency to pulmonary tissue through the use of intermittent oxygen inhalation, antibiotics, postural drainage, and bronchodilators. Abdominal belts, operations on the costal cartilages, and compressed air cabinets all have been used in attempts to improve the mechanical efficiency of the thoracic cage.

Pneumoperitoneum treatment of chronic pulmonary emphysema was first reported by Reich[1] in 1924. He noted increased diaphragmatic motion and improved respiratory function in patients so treated. Piaggio-Blanco[2] in 1937 reported clinical improvement in patients with chronic pulmonary emphysema treated with pneumoperitoneum. His clinical results were not supported by physiologic studies. We have investigated pneumoperitoneum treatment of pulmonary emphysema with the use of well-standardized tests of pulmonary function developed in recent years.

SELECTION OF PATIENTS AND CLINICAL METHODS

From a large group of patients with a chief complaint of dyspnea, thirteen were selected in whom the clinical diagnosis of pulmonary emphysema was confirmed by pulmonary physiologic studies. All patients were hospitalized for clinical evaluation, and endobronchial disease, cardiac decompensation, and active tuberculosis were excluded. Optimal improvement of bronchial infection was obtained prior to first physiologic studies. Patients with predominating pulmonary fibrosis or bronchial asthma were excluded by study in the physiologic laboratory. Dyspnea was so severe in all thirteen patients selected that none were able to work and several were bedridden, requiring intermittent oxygen therapy. Cough and the production of large amounts of sputum were also prominent complaints. No evidence was found to support the diagnosis of cor pulmonale in any case. A diagnosis of arteriosclerotic or hypertensive heart disease was made in four patients and each gave a history of at least one episode of failure treated by digitalis. Except for a history of asthma in three patients, no etiologic factors were found for the emphysema. Laboratory findings on the blood and urine were within normal range.

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PHYSIOLOGIC STUDIES

Some subjective improvement without actual change in pulmonary function was expected as a result of the attention incident to hospital admission. A routine of pulmonary physiologic studies was therefore established to obtain objective data of the ventilatory and respiratory status before and after pneumoperitoneum treatment. The variety of physiologic studies performed was limited by the endurance and cooperation of these very ill patients. The most significant omissions were determinations of alveolar air, gas exchange studies during exercise, and arterial blood gas analyses, although the latter were carried out in a few instances. Bronchospirometry was done only if other studies suggested nonsymmetrical pulmonary insufficiency.

METHOD

Vital capacity and subdivisions were determined by a modified Benedict-Roth recording spirometer. No special effort was made to reduce the resistance of the apparatus because we were only interested in relative changes after treatment. The nomenclature first proposed by Hutchinson[3] was used. The subdivisions were (1) tidal air, or volume of air moved during quiet respiration, (2) complementary air, or maximal volume of inspired air measured from the pulmonary midposition, (3) reserve air, or maximal volume of expired air measured from the pulmonary midposition, and (4) residual air, the volume remaining in the lungs after maximal expiration.

Residual air and lung volume were determined by both the Christie oxygen dilution method without forced breathing[4] and the Darling open circuit method.[5] The latter was found to be more reliable for this type of patient. Lung volumes were obtained by the addition of the vital capacity to the residual air. The predicted vital capacity and lung volume were calculated from the patient's age, height, and sex.[6]

Maximum breathing capacity, defined as the largest volume of air which can be moved in and out of the lungs during a given time,[7] was determined for periods of thirty seconds and calculated to liters per minute at 37° C. The air was collected by means of a Hans Rudolph high-velocity respiratory valve and a Douglas bag.[8] The predicted maximum breathing capacity was calculated from the patient's age, sex, and body surface.[6]

Oxygen consumption, minute ventilation, and ventilatory equivalent were calculated from the spirometric tracings. The ventilatory equivalent is defined as the number of liters of air ventilated for 100 c.c. of oxygen absorbed.[9]

Walking ventilation was determined for those patients able to perform the test. Exhaled air was collected with a respiratory valve and Douglas bag while patients walked over a level course at the rate of 180 feet per minute.[8] Results were obtained from the air collected during the second, third, and fourth minutes of this exercise and were reported in liters per minute at 37° C. The walking ventilation value in normal subjects in our laboratory was found to be 10 liters ± 1 liter per square meter of body surface.

The walking index was calculated from the formula

$$\frac{\text{Walking ventilation}}{\text{Maximum breathing capacity}} \times 100,$$

[8] This index expressed the percentage of the maximum breathing capacity the patient used while walking at the standard rate.

Evaluation of bronchospasm was performed in the laboratory in addition to careful clinical observation for signs of asthma. After satisfactory tests of maximum breathing capacity and vital capacity were obtained, 1 c.c. of 1:200 isopropyl-norepinephrine[10] was given by oxygen aerosol. Resulting improvement of maximum breathing capacity was considered more significant than increase of vital capacity.[11] An increase in performance up to 10 per cent was thought to be within the normal range.

Bronchospirometry was indicated in only one case. Details of this technique have been described by others.[6, 12]

Fluoroscopy was performed on every patient. Maximal diaphragmatic and thoracic cage motion was estimated in per cent of predicted normal.[8] Motion and position of the mediastinum were noted. The lung fields were observed for localized "trapping" of air and the extent of pneumoperitoneum was evaluated. Fluoroscopy was carried out by the same observer before and after institution of treatment.

METHOD OF TREATMENT

Pneumoperitoneum was induced while the patients were still in the hospital, using a standard technique similar to that of Banyai[13] and described in detail elsewhere.[14] Bimonthly refills of 300 to 1,000 c.c. of air maintained intraperitoneal pressures ranging from plus 6 to plus 10 cm. of water. Pneumoperitoneum was maintained for a minimum of six weeks in all patients and was continued to the present writing in eight patients.

RESULTS AND DISCUSSION OF PHYSIOLOGIC STUDIES

Vital capacity determinations were made in the supine and upright positions. A "combined vital capacity" was calculated by the mathematical addition of the volume of reserve air to that of the complementary air, both obtained from spirometric tracings.[6] This was larger in most cases than the direct vital capacity because patients with pulmonary emphysema could empty their lungs more completely if the expiratory effort was not preceded by a maximal inspiration. (Table I.) Throughout this study the "combined vital capacity" in the supine position was used for comparison with other pulmonary function tests as well as for calculation of total capacity.

TABLE I. VITAL CAPACITY BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

	VITAL CAPACITIES IN LITERS AND PER CENT OF PREDICTED											
	UPRIGHT				SUPINE				COMBINED SUPINE			
	BEFORE		AFTER		BEFORE		AFTER		BEFORE		AFTER	
PATIENT	L.	%	L.	%	L.	%	L.	%	L.	%	L.	%
1	2.50	69	3.12	86	2.10	58	2.60	72	2.26	62	2.85	79
2	2.48	65	2.38	62	2.45	64	2.45	64	2.81	73	2.72	71
3	1.92	50	1.92	50	1.90	50	1.90	50	2.20	58	2.28	60
4	0.99	39	1.66	66	0.99	39	1.50	60	1.10	44	1.57	65
6	1.88	51	2.10	57	1.80	49	2.10	57	1.80	49	2.17	59
7	2.28	63	2.26	62	2.28	63	2.26	62	2.48	69	2.35	65
8	2.03	57	2.47	69	1.03	29	1.91	54	1.28	36	1.92	54
9	2.28	62	2.61	71	1.70	45	2.20	58	1.99	54	2.59	70
10	1.24	64	1.45	75	1.17	60	1.43	74	1.41	73	1.55	80
11	2.68	74	2.74	76	2.01	56	2.31	64	2.32	64	2.74	76
12	2.37	71	2.45	73	1.92	57	2.12	64	1.92	57	2.26	68
13	3.30	90	3.85	105	1.95	53	2.39	65	2.58	71	3.18	87
Average	2.08	63	2.43	71	1.77	52	2.10	62	2.02	59	2.35	70

The vital capacity before treatment was smaller than normal in all patients but was larger than anticipated from the severity of their dyspnea.[11, 15] The predicted vital capacity was calculated according to formula and the observed values were expressed as "per cent of predicted." The mean per cent of predicted value for the thirteen patients was 59.2 with a range of 36 per cent to 73 per cent. (Table I.)

TABLE II. TOTAL CAPACITY AND SUBDIVISIONS BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

PATIENT	TIDAL AIR (C.C.)		COMPLEMENTARY AIR (C.C.)		RESERVE AIR (C.C.)		VITAL CAPACITY (C.C.)		TOTAL CAPACITY (C.C.)		RESIDUAL AIR (C.C.)		$\frac{\text{RESIDUAL AIR}}{\text{TOTAL CAPAC.}} \times 100$	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
1	360	340	1,780	2,330	480	520	2,260	2,850	5,710	5,600	3,450	2,750	60.4	49.1
2	280	300	1,845	1,900	590	570	2,455	2,470	5,260	5,190	2,805	2,720	53.3	52.3
3	365	380	1,615	1,680	585	600	2,200	2,280	4,900	4,720	2,700	2,440	55.1	51.7
4	300	280	340	870	700	700	1,100	1,570	3,910	3,700	2,810	2,130	71.8	57.6
6	310	305	1,320	1,785	480	375	1,800	2,170	4,330	4,010	2,530	1,840	58.3	45.8
7	340	350	880	1,250	1,600	1,100	2,480	2,350	4,600	4,400	2,120	2,050	46.1	46.7
8	325	400	905	1,520	375	400	1,280	1,920	4,240	3,600	2,960	1,680	69.8	46.4
9	435	490	1,635	2,090	350	500	1,985	2,590	4,625	3,600	2,640	1,010	57.2	28.0
10	270	300	1,115	1,250	290	300	1,405	1,550	2,925	2,500	1,520	950	51.9	38.0
11	475	460	2,005	2,160	515	580	2,320	2,740	–	–	–	–	–	–
12	380	430	1,305	1,510	610	750	1,915	2,260	5,140	4,600	3,225	2,340	62.9	50.9
13	325	430	1,805	2,390	770	790	2,575	3,180	5,260	5,050	2,685	1,870	51.1	37.0
Average	347	372	1,388	1,728	617	599	1,989	2,345	4,627	4,270	2,577	1,980	58.0	45.8

After treatment with pneumoperitoneum, the vital capacity increased in ten patients and did not change significantly in three. The mean per cent of predicted increased from 59.2 to 69.5, with values ranging from 54 to 87 per cent of predicted. (Tables I and II, Fig. 1.)

The *tidal air,* which was slightly reduced before treatment, did not significantly change after treatment. (Table II, Fig. 1.)

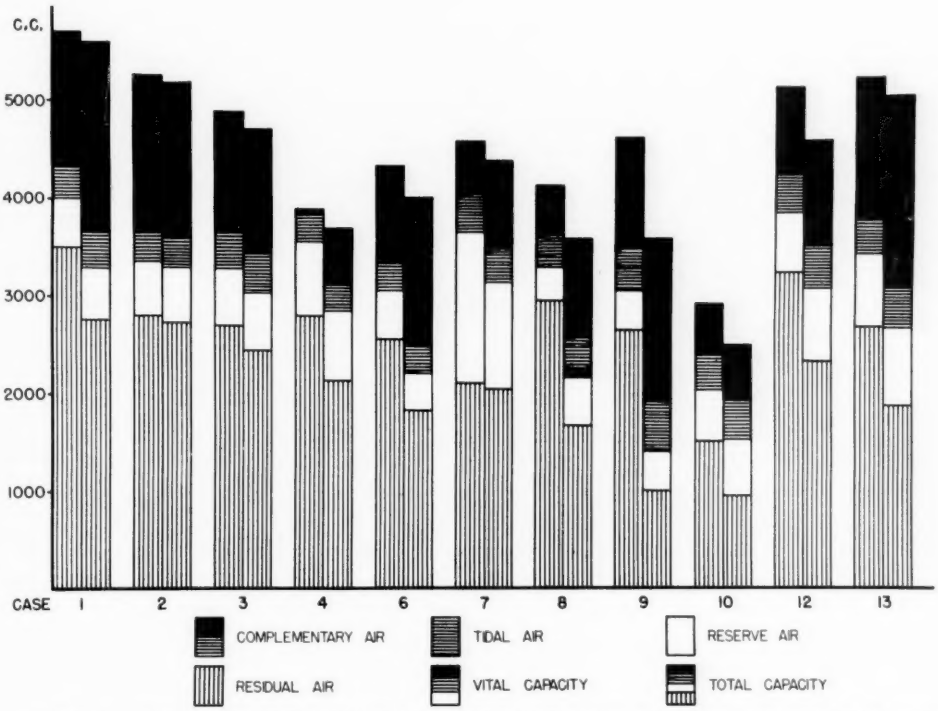

Fig. 1.—Total pulmonary capacity and subdivisions before and after treatment with pneumoperitoneum. The first column (left) represents values before treatment and the second column (right) values after treatment.

The *reserve air* was greatly reduced before treatment in all but one patient. This component was not changed after treatment. (Table II, Fig. 1.)

The *complementary air,* which was severely reduced before treatment, was increased by pneumoperitoneum in every instance. The improvement from a mean of 1,388 c.c. before treatment to a mean of 1,728 c.c. after treatment amounted to 24 per cent, a more consistent and relatively greater increase than that of vital capacity.

Residual air, which is the most graphic index of emphysema, is best expressed as per cent of total lung capacity $\left(\frac{\text{Residual air}}{\text{Total capacity}} \times 100\right)$. In this age group, normal values range from 23 to 30 per cent; 30 to 35 per cent indicates slight emphysema, 35 to 45 per cent denotes moderate, 45 to 55 per cent advanced, and over 55 per cent far advanced emphysema.[16] In our patients, the volume of residual air varied from 1,520 to 3,450 c.c., or from 45.1 to 71.8 per

cent of total capacity. (Table II, Figs. 1 and 2.) Residual air was determined before treatment in eleven patients and, by this classification, four of these had advanced and seven had far advanced pulmonary emphysema.

A dramatic reduction in the volume of residual air was found in all patients after treatment with pneumoperitoneum. When considered by the classification described, five patients improved from the far advanced to the advanced group, two patients from advanced to moderate, and one from far advanced to slight emphysema. (Fig. 2.) There appeared to be no relationship between the initial

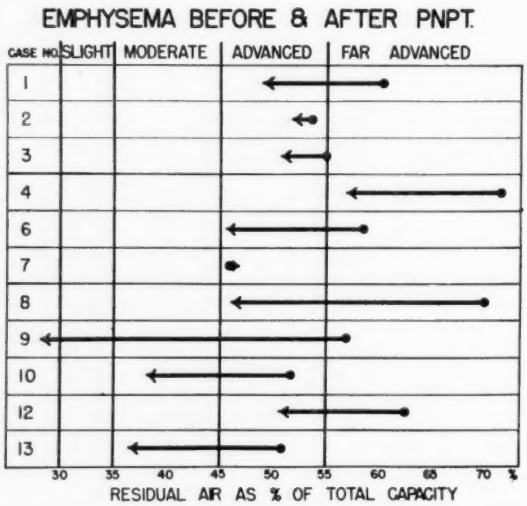

Fig. 2.—Pulmonary emphysema before and after treatment with pneumoperitoneum. The severity of the emphysema is expressed by the ratio $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$.

severity of the disease and the extent of improvement as expressed by reduction of residual air. The mean residual air volume was reduced from 2,677 c.c. to 1,980 c.c., and the mean $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ fell from 58.0 per cent to 45.8 per cent. (Table II, Figs. 1 and 2.)

The *total capacity* before treatment varied little from the predicted figure. The mean per cent of predicted was 96.9 with a range of 109 to 82 per cent. After treatment, the mean total capacity decreased to 89.3 per cent of predicted, an average reduction of 8 per cent. (Table II, Fig. 1.) Reduction in the total capacity indicated a loss in volume of residual air not entirely compensated by an increase in complementary air.

The *maximum breathing capacity* provided the most emphatic demonstration of ventilatory impairment. The mean maximum breathing capacity before treatment was only 33.2 per cent of predicted. Furthermore, the predicted value was calculated by means of a formula which, we believe, gives erroneously low normal values particularly for the older age group.[15] The maximum breathing capacity always increased after treatment with pneumoperitoneum (Table

TABLE III. MAXIMUM BREATHING CAPACITY, WALKING INDEX, AND RESTING BREATHING RESERVE BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

PATIENT	MAX. BREATHING CAP. BEFORE L./MIN.	MAX. BREATHING CAP. BEFORE %	MAX. BREATHING CAP. AFTER L./MIN.	MAX. BREATHING CAP. AFTER %	WALKING VENTILATION (L./MIN.) BEFORE	WALKING VENTILATION (L./MIN.) AFTER	WALKING INDEX (%) BEFORE	WALKING INDEX (%) AFTER	BREATHING RESERVE, RESTING (%) BEFORE	BREATHING RESERVE, RESTING (%) AFTER	AIR VELOCITY INDEX % PREDICTED M.B.C. / % PREDICTED V.C. BEFORE	AIR VELOCITY INDEX % PREDICTED M.B.C. / % PREDICTED V.C. AFTER
1	38	42	50	56	19	19	50	38	82.0	87.8	0.68	0.71
2	34	31	40	37	26	24	72	60	65.9	82.0	0.42	0.52
3	22	22	26	26	21	21	95	81	70.2	75.0	0.38	0.42
4	12	24	29	53	Unable	14	100	48	33.2	80.7	0.54	0.82
5	30	35	34	40	Unable	Unable	53	47	59.2	76.5	0.56	0.53
6	29	32	31	34	Unable	16	55	52	78.6	80.6	0.65	0.58
7	26	27	34	34	13	13	50	38	65.0	75.3	0.39	0.52
8	25	27	37	39	19	19	76	51	57.2	82.7	0.75	0.72
9	33	39	46	54	17	16	52	35	76.3	85.1	0.72	0.77
10	19	36	24	45	13	12	69	50	65.9	76.3	0.49	0.56
11	44	46	47	49	23	17	52	36	81.6	83.4	0.72	0.65
12	22	26	28	33	18	14	82	50	67.2	73.9	0.39	0.49
13	38	43	53	60	18	18	47	34	84.6	87.8	0.60	0.69
Average	29	33	37	43	19	18	66	48	68.2	80.5	0.56	0.62

III). Increments ranged from 2 to 15 liters per minute, an average improvement of 33 per cent. However, even after treatment, all patients still showed less than half their expected normal maximum breathing capacity (Table III).

The *air velocity index* $\left(\frac{\text{Per cent of predicted maximum breathing capacity}}{\text{Per cent of predicted vital capacity}}\right)$ is a numerical expression of air flow velocity.[15] Normal values range from 1.0 to 1.2 with the prediction formulas used by us. A relatively greater reduction of maximum breathing capacity than of vital capacity results in an index less than 1.0. This indicates a relatively large pulmonary capacity with inability to move air in and out of the chest rapidly.

Before treatment, the mean air velocity index was 0.56, evidence of loss of pulmonary elasticity and of the great obstructive element in emphysema. In other words, the vital capacity was relatively much larger than the maximum breathing capacity. After treatment with pneumoperitoneum, there was a small increase of the mean index to 0.62 (Table III). Both vital capacity and maximum breathing capacity increased, but there was a greater increase of the latter. Nevertheless, after treatment, maximal air flow velocity remained seriously reduced.

Walking ventilation was obtained in ten patients before treatment. Severe dyspnea precluded walking at the required rate for two patients, and a third was paraplegic. All ten patients were severely dyspneic after this mild exercise test. Two patients showed an abnormally high walking ventilation before treatment. After treatment all but the paraplegic patient were able to perform the test satisfactorily and only five showed any evidence of dyspnea.

A decrease in walking ventilation was observed in one-half of the patients while it remained unchanged in the others (Table III). This decrease may have been due to an increase in the efficiency of alveolar mixing, a decreased tendency to hyperventilation, or a combination of both.

The *walking index* before treatment was over 50 per cent in all but one patient (Table III). Classified by this index, all but one patient fell into the severely dyspneic group.[8] After treatment, only four patients remained in this group while five patients were in the "slight or no dyspnea" group. The mean walking index decreased from 65.8 per cent before treatment to 47.7 per cent after treatment. This reduction was due to a decrease in walking ventilation in some patients, in addition to an increase in the maximum breathing capacity present in all patients.

Minute oxygen consumption per square meter of body surface was elevated in all but three patients, suggesting that the patients were not in a basal state when tested. After treatment, the oxygen consumption remained within 12 c.c. of the pretreatment value, indicating identical testing conditions before and after treatment (Table IV).

TABLE IV. VENTILATION, OXYGEN CONSUMPTION, VENTILATORY EQUIVALENT, AND RESPIRATORY RATE AT REST BEFORE AND AFTER PNEUMOPERITONEUM TREATMENT

	MINUTE VENTILATION PER SQ. M. BODY SURFACE (L.)		MINUTE OXYGEN CONSUMPTION PER SQ. M. BODY SURFACE (C.C.)		VENTILATORY EQUIVALENT (L. PER 100 C.C. OXYGEN)		BASAL RESPIRATORY RATE PER MIN.	
PATIENT	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
1	4.17	3.73	140	134	3.0	2.8	19	18
2	6.56	4.07	154	160	4.2	2.5	36	24
3	3.69	3.65	114	115	3.2	3.2	18	17
4	5.47	3.52	173	224	3.4	1.6	29	20
6	4.37	3.86	148	148	2.9	2.6	20	18
7	5.58	5.15	187	196	3.0	2.6	27	24
8	6.19	3.93	164	168	3.8	2.3	31	16
9	5.14	4.52	151	138	3.4	3.0	18	14
10	5.23	4.59	145	133	3.6	3.4	24	19
11	4.53	4.39	205	198	2.2	2.2	17	17
12	4.88	4.94	127	135	3.9	3.6	19	17
13	3.44	3.80	124	132	2.8	2.9	18	15
Average	5.14	4.24	153	157	3.3	2.7	23	18

Minute ventilation per square meter of body surface was increased in all but two patients and significantly increased in five. After treatment there was a mean reduction of 18 per cent (Table IV).

The *ventilatory equivalent* was moderately elevated in twelve of the thirteen patients. A mean ventilation of 3.28 liters of air for 100 c.c. of oxygen absorbed indicated an increase of about 50 per cent over the normal 2.2 liters (Table IV). This elevation may have been due to hyperventilation at rest. It was more likely an indication of alveolorespiratory insufficiency in the form of poor alveolar mixing, an increased alveolocapillary threshold, or both. After treatment, the mean ventilatory equivalent decreased to 2.72 liters (Table IV). The fact that the equivalent could be reduced by mechanical reduction in the volume of residual air suggests inefficient alveolar mixing as the chief cause for the elevated equivalent before treatment.

The *resting breathing reserve* was invariably reduced. The largest reserve found in this group was 84.6 per cent and the lowest 33.2 per cent, compared

with a normal reserve of 92 per cent or more.[6] The patient with the lowest reserve used two-thirds of her best ventilatory effort merely for ventilation at rest. Pneumoperitoneum caused a mean increase of the breathing reserve of 18 per cent. After treatment, the group of patients used only 19.5 per cent of their best effort for resting ventilation compared with 31.8 per cent before treatment (Table III). This reflects both an increased maximum breathing capacity and a decreased resting minute ventilation.

Arterial blood gas analysis was performed in three cases. Hemoglobin oxygen saturation was below normal at rest and greatly reduced after exercise. Carbon dioxide tension was elevated at rest and respiratory acidosis developed in all three patients after exercise (Table V).

TABLE V. RESPIRATORY GASES IN THE ARTERIAL BLOOD BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

PATIENT		OXYHEMOGLOBIN SATURATION (%)		CARBON DIOXIDE TENSION (MM. HG)	
		BASAL	AFTER EXERCISE	BASAL	AFTER EXERCISE
3	Before treatment	88.2	64.2	46.0	60.2
	After pneumoperitoneum	87.0	64.8	48.4	60.0
4	Before treatment	90.0	67.2	42.4	54.6
	After pneumoperitoneum	92.8	88.6	40.0	42.4
7	Before treatment	84.2	58.8	51.0	62.0
	After pneumoperitoneum	88.0	75.6	44.2	—

After treatment with pneumoperitoneum, Patient 3 showed no change of resting oxygen saturation and carbon dioxide tension, and improvement after exercise was very small. Patient 4 showed a definite improvement of resting oxygen saturation from 90.0 to 92.8 per cent and of carbon dioxide tension from 42.4 to 40.0 mm. Hg. Improvement of postexercise values was dramatic. Oxygen saturation increased from 67.2 to 88.6 per cent and carbon dioxide tension decreased from 54.6 to 42.4 mm. Hg. This is more remarkable since pretreatment exercise in this patient was less than the standard exercise test. Patient 7 improved from a postexercise oxygen saturation of 58.8 per cent to a saturation of 75.6 per cent (Table V).

Bronchospasm was demonstrated in two patients by a 20 per cent increase in maximum breathing capacity though five other patients showed slight but not abnormal increases. Rise in vital capacity was smaller and bore no relationship to change in maximum breathing capacity. The mean rise of vital capacity after aerosol was 5 per cent; the mean rise for the maximum breathing capacity was 8 per cent.

After induction of pneumoperitoneum, the increase of maximum breathing capacity following bronchodilator aerosol was more pronounced, averaging 11 per cent. Patients unimproved before pneumoperitoneum continued to show no increase. Small bronchodilator increases before treatment became much more marked in three patients after pneumoperitoneum (Table VI). This suggests

TABLE VI. BRONCHOSPASM STUDIES BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

PATIENT	MAXIMUM BREATHING CAPACITY (L./MIN.)				UPRIGHT VITAL CAPACITY (L.)			
	BEFORE PNEUMOPERITONEUM		AFTER PNEUMOPERITONEUM		BEFORE PNEUMOPERITONEUM		AFTER PNEUMOPERITONEUM	
	BEFORE AEROSOL	AFTER AEROSOL	BEFORE AEROSOL	AFTER AEROSOL	BEFORE AEROSOL	AFTER AEROSOL	BEFORE AEROSOL	AFTER AEROSOL
1	38	38	50	50	2.50	2.56	3.12	3.21
2	34	41	40	48	2.48	2.60	2.38	2.60
3	22	21	26	26	1.92	1.90	1.92	1.90
4	13	13	29	27	0.99	1.30	1.66	1.60
8	25	28	37	45	2.03	2.46	2.47	2.58
10	19	17	24	24	1.24	1.20	1.45	1.47
11	44	53	47	59	2.68	2.86	2.74	2.80
12	22	23	28	41	2.37	2.35	2.45	2.45
13	38	41	53	56	3.30	3.29	3.85	4.12
Average	28	31	37	42	2.17	2.28	2.45	2.53

that impairment of ventilation caused by spasm was previously masked by the limiting effect of emphysema but, after treatment with pneumoperitoneum, became more noticeable as the emphysema was mechanically diminished. Changes in vital capacity with aerosol continued to be insignificant after treatment with pneumoperitoneum.[11, 15]

Fluoroscopy demonstrated symmetrical reduction of thoracic cage motion in twelve patients through an estimated range of 10 to 50 per cent of normal. Diaphragmatic motion was symmetrically reduced in every case to an average of 10 per cent of normal. Three patients showed marked paradoxical descent of the diaphragm during forced expiration. In one patient there was a large cystlike area devoid of lung markings at the base of one lung with marked trapping of air (Table VII).

TABLE VII. FLUOROSCOPIC FINDINGS BEFORE AND AFTER TREATMENT WITH PNEUMOPERITONEUM

PATIENT	THORACIC CAGE MOTION (% OF NORMAL)				DIAPHRAGMATIC MOTION (% OF NORMAL)				PNEUMOPERITONEUM (0 TO ++++)	
	BEFORE		AFTER		BEFORE		AFTER		AFTER TREATMENT	
	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT
1	25	25	25	25	−5	−5	25	25	++	++
2	25	25	30	30	15	15	35	35	+	+
3	10	10	10	10	25	25	10	40	+	+
4	10	10	10	10	10	10	50	50	++	++
5	20	20	20	20	10	10	35	35	+	+
6	10	10	10	10	20	0	30	10	+	+
7	25	25	25	25	10	10	40	30	+	+
8	30	60	50	75	20	20	30	65	++	++
9	30	30	30	30	10	10	35	15	+	+
10	50	50	50	50	−5	−5	30	30	+	+
11	40	40	40	40	30	30	60	60	++	++
12	10	10	10	10	30	30	75	75	++	++
13	30	30	40	40	−25	−25	80	80	++	++
Average	24	27	27	29	11	10	41	42		

The pneumoperitoneum used in this series was small when compared with the customary pneumoperitoneum for the treatment of tuberculosis. It varied from a rim of air without elevation of the diaphragm on deep inspiration to a moderate pneumoperitoneum with a rise of the diaphragm of one-half interspace.

Motion of the diaphragm was increased from an average of 10 per cent of normal before treatment to 42 per cent of normal after treatment with pneumoperitoneum. There was some relationship between the extent of the pneumoperitoneum and the improvement of diaphragmatic motion. No relationship was found between the extent of the initial impairment of motion and the degree of improvement. Diaphragmatic motion increased symmetrically in all patients except one with a large bleb in addition to severe emphysema in whom movement was actually less after treatment on the side immediately below the bleb. Fluoroscopy after induction of pneumoperitoneum revealed no significant change in the range of motion of the thoracic cage (Table VII).

Bronchospirometry was performed on the patient with the large bleb. The relative oxygen consumption of that side (right) was reduced to 48 per cent of the total while minute ventilation and vital capacity were 59 per cent of the total. The bleb apparently aerated but did not contribute to oxygen consumption. There was no change after treatment.

RELATIONSHIP OF IMPROVEMENT TO DURATION OF TREATMENT

In addition to the early routine studies, one patient was restudied 94 days and a second 167 days after beginning treatment (Table VIII, Fig. 3). In both cases there was clinical as well as laboratory evidence of improved respiratory function shortly after initiation of treatment. The later studies demonstrated a further improvement of ventilatory function and a further decrease of residual air. Concomitantly, further increase of the pneumoperitoneum was observed. These studies suggest that full realization of the clinical and laboratory results

TABLE VIII. RELATIONSHIP OF IMPROVEMENT TO DURATION OF TREATMENT WITH PNEUMOPERITONEUM

	PATIENT NO. 4			PATIENT NO. 8		
	DAYS AFTER TREATMENT					
	0	17	167	0	13	94
Tidal air (c.c.)	300	314	280	325	330	400
Complementary air (c.c.)	340	680	870	905	1,030	1,520
Reserve air (c.c.)	760	740	700	375	370	400
Vital capacity (c.c.)	1,100	1,420	1,570	1,280	1,400	1,920
Total capacity (c.c.)	3,910	3,800	3,700	4,240	4,100	3,600
Residual air (c.c.)	2,810	2,380	2,130	2,960	2,700	1,680
$\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ (%)	71.8	62.7	57.6	69.8	65.8	46.4
Ventilatory equivalent (L.)	3.4	2.8	1.6	3.8	2.9	2.3
Maximum breathing capacity (L./min.)	13	26	29	25	33	37
Walking ventilation (L.)	Unable	15	14	19	19	19
Walking index (%)	—	57	48	76	58	51
Motion of diaphragms (%)	10	30	50	10	15	25
Pneumoperitoneum (0 to 4+)	0	+	++	0	+	++

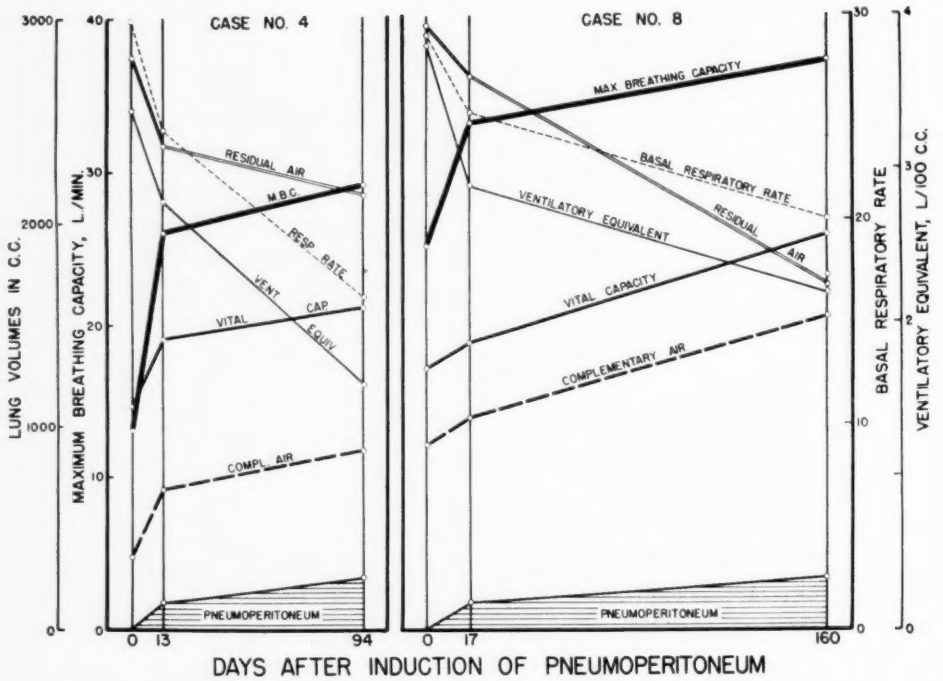

Fig. 3.—Duration of pneumoperitoneum treatment related to improvement of pulmonary emphysema. Te patient illustrated on the left (Case 4) was studied before treatment, thirteen days after induction of pneumoperitoneum, and ninety-four days after induction. The patient illustrated on the right (Case 8) was studied before treatment and seventeen and one-hundred-sixty days after beginning of treatment.

of pneumoperitoneum treatment cannot be obtained shortly after beginning of treatment (Table VIII, Fig. 3).

EVALUATION OF CLINICAL RESULTS

Ten of the thirteen patients reported a decrease in severity of their symptoms following treatment. This was characterized by lessened dyspnea, increased exercise tolerance, better sleep, and improved tussive force. Improvement sometimes immediately followed the initial injection of air. Maximum clinical benefit was commonly not apparent for at least one month. Although two patients felt that pneumoperitoneum did not improve their major symptoms, they nevertheless were able to cough much more forcefully. The patient with a large bleb showed no improvement.

CORRELATION OF CLINICAL RESULTS WITH PHYSIOLOGIC STUDIES

The laboratory demonstration of successful treatment of chronic emphysema should show an improved ventilatory status as expressed by an increase in maximum breathing capacity, vital capacity, and complementary air. Improved intrapulmonary mixing should be demonstrated by a decrease in residual air, basal respiratory rate, and ventilatory equivalent. Clinically, a decrease of dyspnea, cough, expectoration, and cyanosis and an increase of exercise tolerance,

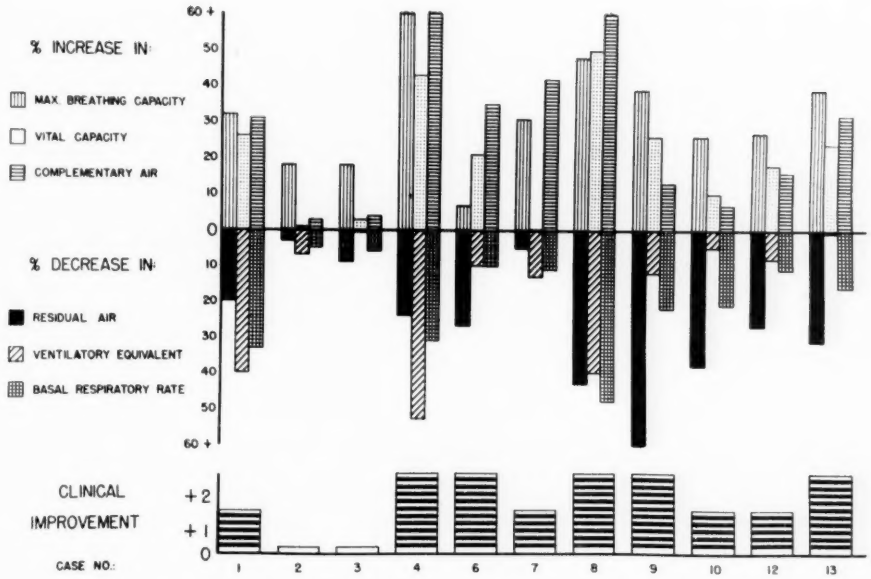

Fig. 4.—Correlation of clinical status with laboratory data; patients with pulmonary emphysema treated with pneumoperitoneum. Clinical improvement was rated as 0 (unimproved), plus 1 (moderate improvement), and plus 2 (excellent improvement). Maximum breathing capacity, total capacity and subdivisions, ventilatory equivalent, and basal respiratory rate are expressed as per cent increase or decrease.

TABLE IX. CORRELATION OF PHYSIOLOGIC AND CLINICAL RESULTS

	PATIENT										
	1	2	3	4	6	7	8	9	10	12	13
Evidence of Improved Ventilation											
% Increase in maximum breathing capactiy	32	18	18	108	7	31	48	39	26	27	39
% Increase in vital capacity	26	1	3	43	21	-5	50	26	10	18	24
% Increase in complementary air	31	4	4	164	35	42	68	13	7	16	34
% Increase in diaphragmatic motion	30	20	0	40	20	25	25	15	35	45	105
Evidence of Improved Alveolar Mixing											
% Decrease in residual air	20	3	9	24	27	5	43	62	38	27	31
% Decrease in ventilatory equivalent	40	7	0	53	10	13	49	12	5	8	0
% Decrease in respiratory rate	33	5	6	31	10	11	48	22	21	11	16
% Decrease of walking ventilation	0	8	0	-	-	0	0	0	6	22	10
% Decrease of minute ventilation	11	38	1	36	12	8	36	12	12	0	0
Evidence of Improved Respiratory Reserve											
% Decrease of walking index	24	17	15	-	-	24	33	33	28	39	28
% Increase of breathing reserve	7	24	7	142	2	16	45	11	11	10	5
Evidence of Clinical Improvement											
Less dyspnea and cyanosis, greater exercise tolerance (0 to ++)	++	0	0	++	+	0	++	++	++	+	++
More effective cough, less sputum (0 to ++)	++	+	0	++	+	+	++	+	+	+	++

effectiveness of cough, and motion of the diaphragm by fluoroscopy should be observed.

An attempt was made to correlate the evidence of successful treatment. In Table IX and Fig. 4 physiologic changes, expressed as per cent increase or decrease, are related to clinical improvement classified as excellent (plus 2), moderate (plus 1), or none (0). Two patients were not clinically improved (Patients 2 and 3). These two also showed no laboratory evidence of improved respiratory status except for a slight increase in maximum breathing capacity. In one of them (Patient 2) severe pulmonary fibrosis was present and several giant blebs complicated the pulmonary emphysema of the other (Patient 3). Two subjects (Patients 4 and 8) who showed the most dramatic improvement in every respect were the ones restudied some time after induction of pneumoperitoneum (Table IX, Fig. 4). In other cases the correlation between physiologic data and clinical improvement was equally good.

SUMMARY

The pulmonary functions and volumes of thirteen patients with far-advanced pulmonary emphysema were studied before and after treatment with pneumoperitoneum.

Marked changes were observed in several subdivisions of the total pulmonary capacity. The complementary air was always enlarged, with a mean increase of 24 per cent; residual air was invariably reduced, with a mean reduction of 26 per cent; tidal air and reserve air were not significantly changed. The vital capacity was increased in ten patients and the total capacity was somewhat reduced in all.

The maximum breathing capacity was increased by a mean of 33 per cent, while ventilation at rest and during standard exercise was reduced. Consequently, the breathing reserve was increased from 68.2 per cent before treatment to 80.5 per cent after treatment.

Minute oxygen consumption remained unchanged and the ventilatory equivalent was reduced from 3.28 to 2.72 liters per 100 c.c. of oxygen consumed.

In two patients studied by blood gas analysis, treatment resulted in a slight increase in hemoglobin oxygen saturation at rest, a marked increase after exercise, and a corresponding reduction in arterial carbon dioxide tension.

Bronchospasm was greater in three patients after treatment.

Fluoroscopy demonstrated a marked increase in diaphragmatic motion after treatment.

Improvement increased with lengthened time of treatment.

Clinical improvement was characterized by lessened dyspnea, increased exercise tolerance, better sleep, and increased tussive force.

A high degree of correlation was shown between clinical improvement and laboratory evidence of improved pulmonary function. Reduction of residual air appeared to be the most significant finding.

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BLOOD ERGOTHIONEINE LEVELS IN DIABETES MELLITUS

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INTEREST in blood ergothioneine, a naturally occurring erythrocyte constituent, was renewed with the report of Lawson and Rimington[1, 2] in 1947 which attributed an antithyroid property to it. Stanley and Astwood[3] and Wilson and McGinty[4] made attempts to demonstrate an antithyroid effect. Both groups used newer radioactive iodine techniques, but they were unable to confirm the earlier work of the London group.

Little work has been reported on its relationship to disease states. Salt,[5] in 1931, reported slightly elevated blood ergothioneine levels in seventeen patients with diabetes and single cases of rheumatism, pulmonary tuberculosis, and hemiplegia. Benedict, Newton, and Behre[6] had made the same observation in diabetes but had given no values.

Only two reports on the pharmacology of ergothioneine have been found. Tainter,[7] in 1927, demonstrated no change in blood glucose levels or other signs of pharmacologic activity in cats following the subcutaneous injection of ergothioneine. An Italian worker, Trabucchi,[8] also considered ergothioneine pharmacologically inactive but thought that it exhibited a mild antiberiberi action.

With the development by Hunter[9, 10] of a modification of his earlier diazo test, and the adaptation of it to the Evelyn colorimeter, it was felt that further investigation into the ergothioneine values in pathologic states was indicated. From the reports cited it appeared that there was no relationship between ergothioneine and thyroid function. Since slightly elevated levels of blood ergothioneine had been reported in diabetes, this disease was chosen for study.

METHODS AND MATERIALS

Blood for estimation in diabetes was obtained from patients admitted to two general hospitals, either for periodic checks or because of a diabetic complication. The samples were portions of oxalated blood taken by the hospital laboratory staff for blood glucose estimations. Blood from normal persons was obtained from the University student population and from donors at the Red Cross Blood Donor Clinic, blood from the latter being taken at the conclusion of the donation.

The method for estimation was that described by Hunter[10] in 1949, with one modification. It was found that the plasma, which Hunter stated gave a yellow color with the diazo test, contributed to the colorimetric value measured as ergothioneine in whole blood. The plasma value was independent of the amount of ergothioneine present and proportional to the hematocrit reading for the plasma in any specimen. It was found that the color developed by pure plasma gave an "ergothioneine-equivalent" value of 1.0 mg. per cent ± 0.2.

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A fraction of an average value of 1.0 mg. per cent, proportional to the percentage of plasma found in each specimen, was subtracted from the ergothioneine value obtained for the whole blood in the same specimen. This then gave the true ergothioneine reading.

Different dilutions of cells with plasma were made from one blood, and the foregoing method of calculation for true ergothioneine content was found to be valid for all hematocrit values encountered. (Table I.) This plasma correction is especially significant when the hematocrit is low, since the difference is increased to a greater extent on conversion of the

TABLE I. THREE DIFFERENT CORPUSCLE-PLASMA DILUTIONS FROM THE SAME BLOOD

ERGOTHIONEINE (MG. % WHOLE BLOOD)	HEMATOCRIT (%)	ERGOTHIONEINE (MG. % CORPUSCLES) (CALCULATED)	ERGOTHIONEINE (MG. % WHOLE BLOOD LESS PLASMA EFFECT)	ERGOTHIONEINE (MG. % CORPUSCLES LESS PLASMA EFFECT)
2.5	20	12.5	2.5 −(80% of 1) = 1.7	8.5
4.0	40	10.0	4.0 −(60% of 1) = 3.4	8.5
5.5	60	9.2	5.5 −(40% of 1) = 5.1	8.5

ergothioneine reading from whole blood to per cent corpuscles. No ergothioneine was found in plasma, and the "ergothioneine-equivalent" readings in plasma remained constant in blood specimens which gave ergothioneine values ranging from 4 to 60 mg. per cent corpuscles. To confirm the belief that all the ergothioneine measured by this method was contained in the corpuscles, estimations were made on whole blood and the values converted by hematocrit to per cent corpuscles, estimations were then made on the corpuscles alone from the same sample. Two examples of this calculation are given in Table II in which it can be seen that the calculated value of ergothioneine per cent corpuscles using whole blood agrees with the value found on using corpuscles alone.*

If the plasma effect were not taken into consideration, the respective values per cent corpuscles would be 7.8 mg. per cent and 4.8 mg. per cent, readings 1.4 mg. per cent and 1.1 mg. per cent higher than the actual value for corpuscles of 6.4 mg. per cent and 3.7 mg. per cent.

TABLE II. COMPARISON OF ERGOTHIONEINE VALUES FOUND IN CORPUSCLES AND CALCULATED FROM WHOLE BLOOD

ERGOTHIONEINE (MG. % WHOLE BLOOD)	HEMATOCRIT (%)	ERGOTHIONEINE (MG. % CORPUSCLES) (CALCULATED)	ERGOTHIONEINE (MG. % WHOLE BLOOD LESS PLASMA EFFECT)	ERGOTHIONEINE (MG. % CORPUSCLES, FOUND)
3.3	42.5	6.4	3.3 −(57.5% of 1) = 2.7	6.4
2.2	46.0	3.7	2.2 −(54 % of 1) = 1.7	3.7

In the estimation of blood ergothioneine, a bronze color occasionally appears upon the addition of the 10N NaOH to the coupled compound, instead of the usual violet-pink. This prevents the estimation of ergothioneine, and once it appears in a specimen it is constantly present on repeated determinations. This problem has not yet been solved. The only results reported in this paper are from tests in which a normal ergothioneine color developed. All values given were made on whole blood and are reported in milligrams of ergothioneine per 100 ml. corpuscles.

RESULTS

The diabetic group of 107 hospital patients was compared with the normal group of 94 persons. Of the diabetic group, 50.9 per cent were male and 49.1 per cent were female patients; 80.8 per cent of the normal group were male

*To minimize the difficulty experienced in obtaining the corpuscles free from plasma dilution, the sample was first centrifuged at 3,000 r.p.m. and then frozen. The erythrocyte layer was then cut from the frozen core, melted, and carried through the test. Freezing did not affect the test.

and 19.2 per cent were female subjects. A comparison of the two groups is shown in Table III. On statistical comparison of the two means a highly significant difference was found to exist between the normal and the diabetic groups.

It will be seen from Fig. 2 that this difference was caused by diabetic patients who had ergothioneine values greater than 14.5 mg. per cent. No normal

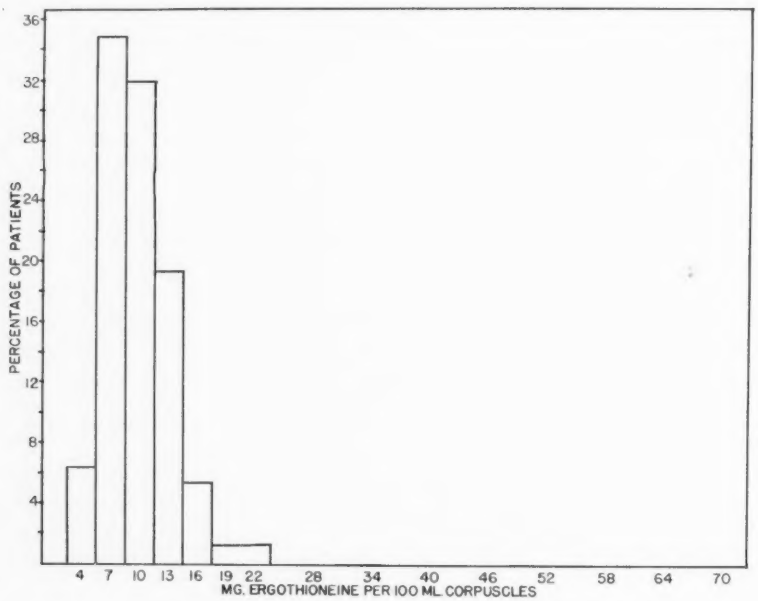

Fig. 1.—Distribution of blood ergothioneine levels in ninety-four normal subjects.

person had a value greater than 17.7 mg. per cent. The range for normal subjects extended from 3.9 to 17.7 mg. per cent and the range for diabetic subjects from 2.0 to 60.2 mg. per cent. Seventeen diabetic patients (15.9 per cent of the total number of diabetics) exceeded the highest normal value.

Because the mean of the age of the normal group was much lower than that in the diabetic series, it was felt that a comparison of ergothioneine levels

TABLE III. ERGOTHIONEINE VALUES IN NORMAL AND DIABETIC BLOOD

	NORMAL	DIABETIC
Number of patients	94	107
Average age (yr.)	29.5	52.1
Average age—male	29.6	52.7
Average age—female	29.1	52.2
Mean ergothioneine value (mg. % corpuscles) for male and female subjects	9.6	12.7
Mean—male	9.7	13.0
Mean—female	9.5	12.2
Standard deviation (all patients)	3.1	8.3
Standard error of mean	0.32	0.80
t value = 3.580		
t value at 1 per cent level = 2.601		

in different age groups in the normal group should be made. It will be seen in Table IV that the ergothioneine value in the 45 to 63 year interval is greater than the mean for the group as a whole. The increase was not progressive throughout the group, however, and on comparison of two approximately equal numbers of patients from the 25 to 29 year interval (thirty-eight patients) and the 30 to 63 year interval (twenty-seven patients) we found no significant difference between the means.

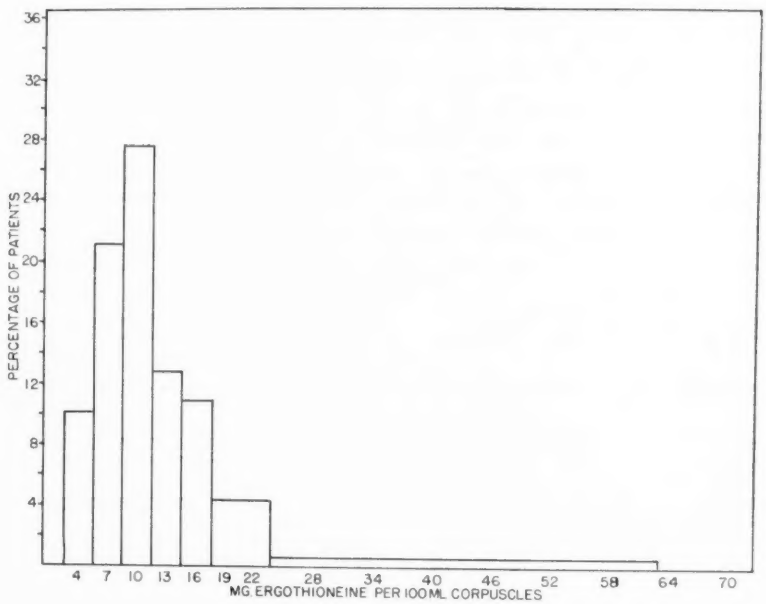

Fig. 2.—Distribution of blood ergothioneine levels in 107 diabetic patients.

TABLE IV. BLOOD ERGOTHIONEINE LEVELS AT DIFFERENT AGE INTERVALS IN NORMAL PERSONS

AGE INTERVAL (YR.)	NUMBER OF PERSONS	ERGOTHIONEINE (MG. % CORPUSCLES)	STANDARD DEVIATION	STANDARD ERROR OF MEAN
15-19	5	8.3	3.83	1.71
20-24	24	9.7	2.56	0.52
25-29	38	9.00	2.28	0.37
30-34	9	11.20	3.07	1.02
35-44	5	7.70	0.89	0.40
45-63	13	11.6	4.22	1.17
25-29	38	9.00	2.28	0.37
30-63	27	10.73	3.66	0.70
$t = 0.659$; t value at 5 % level $= 2.000$				

The diabetic series was then divided into age groups and in contrast to the normal group showed a somewhat lower value in the older age intervals. On comparing the age group 2 to 59 (forty-three patients) with the age group 60 to 89 (thirty-three patients), the difference of the means was not found to be significant.

The state of diabetic control and the duration of diabetes, two factors which might have borne some relation to ergothioneine level in diabetic persons, were

TABLE V. BLOOD ERGOTHIONEINE LEVELS AT DIFFERENT AGE INTERVALS IN DIABETIC PATIENTS

AGE INTERVAL (YR.)	NUMBER OF PATIENTS	ERGOTHIONEINE (MG. % CORPUSCLES)	STANDARD DEVIATION	STANDARD ERROR OF MEAN
2-39	14	14.24	8.42	2.25
40-49	14	12.90	5.68	1.52
50-59	15	17.50	10.48	2.70
60-69	20	12.30	12.09	2.70
70-89	13	8.22	2.73	0.76
0-59	43	14.94	8.31	1.27
59-89	33	10.67	9.67	1.69
$t = 1.15$; t value at 5% level $= 1.994$				

then examined. Table VI shows a comparison of the poorly controlled and the well-controlled diabetic subjects in the series. A history of the state of control was obtained in eighty-two of the 107 patients. A comparison showed that no significant difference existed in ergothioneine levels of well- and poorly controlled diabetic persons. In seventeen diabetic patients previously referred to who had levels higher than the highest normal value of 17.7 mg. per cent, eleven or 64.7 per cent were severely diabetic or were poorly controlled. However, that left six (35.3 per cent) patients with high values who were well-controlled and often mildly diabetic.

TABLE VI. BLOOD ERGOTHIONEINE LEVELS IN WELL-CONTROLLED AND POORLY CONTROLLED DIABETIC SUBJECTS

	POORLY CONTROLLED	WELL CONTROLLED
Number of patients	47	35
Mean ergothioneine level (mg. %)	13.8	12.1
Standard deviation	10.02	6.97
Standard error of mean	1.46	1.18
$t = 0.48$; t value at 5% level $= 1.990$		

A comparison was made between diabetic subjects who had a history of the disease of less than ten years and those who had been diabetic for ten years or longer. The history of duration of diabetes was obtained in sixty-four of the 107 patients. There was no significant difference between the means of the diabetic patients with recent disease and those with long-standing diabetes, as shown in Table VII.

TABLE VII. BLOOD ERGOTHIONEINE LEVELS IN DIABETIC SUBJECTS WITH DISEASE DURATION OF MORE AND LESS THAN 10 YEARS

	DIABETES FOR LESS THAN 10 YR.	DIABETES FOR 10 YR. AND LONGER
Number of patients	39	25
Mean ergothioneine level (mg. %)	12.19	14.28
Standard deviation	7.81	10.96
Standard error of mean	1.25	2.19
$t = 0.506$; t value at the 5% level $= 2.000$		

Two patients, admitted in diabetic coma, with blood glucose levels of over 400 mg. per cent and CO_2 combining powers of 40 vol. per cent, were found to have normal ergothioneine values which did not change on control of their diabetic state.

One patients in insulin shock with a blood glucose of 47 mg. per cent showed an increase of ergothioneine to 17.1 mg. per cent from a previous reading of 6.9 mg. per cent the same day, with a return to the lower level when next tested five days later. Because of this observation, samples of blood were taken from four patients being treated with subshock doses of insulin on a psychiatric service. These were obtained at six-thirty in the morning and again about 9:30 A.M., by which time the symptoms of hypoglycemia from the early morning insulin were maximal. There was no difference in the ergothioneine levels in any pair of specimens.

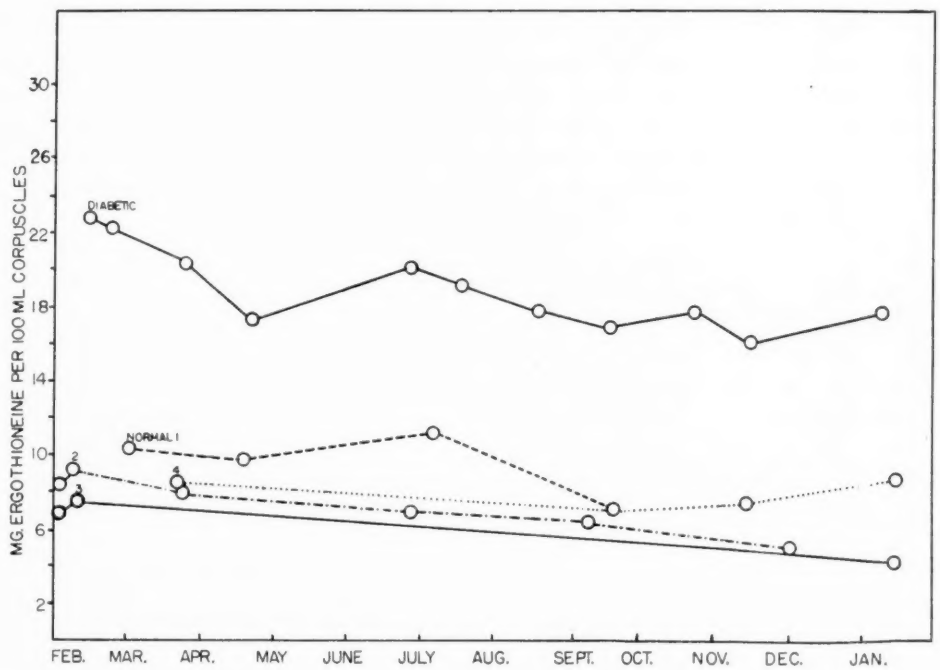

Fig. 3.—Periodic blood ergothioneine levels in one diabetic student and four normal subjects.

In eleven patients from whom samples had been obtained at various times during the same day, usually at 8:00 A.M., 11:00 A.M., and 2:00 P.M., there was no apparent relation to meals or to the time of day at which the specimens were taken.

A diabetic patient examined preoperatively and postoperatively (hip nailing) showed a postoperative rise to 16.1 mg. per cent of ergothioneine from a previous level of 9.4 mg. per cent, and still showed the higher level four days later. No other operative patients were examined.

The lowest value in diabetes, 2.0 mg. per cent, was found in a 68-year-old woman who was controlled on 15 units of protamine zinc insulin and who was also suffering from hypertension and senile psychosis. Another low value of 4.2 mg. per cent was observed in a patient who had a fasting blood glucose of 250 mg. per cent and who was also being treated for cystitis and mild congestive heart failure.

Ergothioneine estimations were made on a well-controlled diabetic student at monthly intervals. The small fluctuations seen in Fig. 3 cannot be explained by any change in his diet, which is necessarily more restricted than that of a normal person, nor by any change in diabetic control, which has been excellent over this period. Similar small fluctuations can be seen in ergothioneine levels of normal subjects taken over shorter periods of time.

Nonprotein nitrogen, uric acid, and blood cholesterol values in twenty-five, thirty-four, and fifty-four patients respectively showed no correlation with the ergothioneine content of the same blood.

DISCUSSION

It is difficult, when assessing such findings, to separate other influences from the diabetes, since many of the diabetic persons were in an older age group and were suffering from the usual degenerative complications. Diabetes has been assumed to be the dominant pathologic state. It will be of interest to investigate other diseases and discover what range of pathologic conditions shows changes in blood ergothioneine values. At present, work is proceeding here in that field and will be the subject of a further report. It remains to be shown whether ergothioneine is entirely exogenous or whether it is an active constituent of the blood. It would seem unlikely that dietary influences would vary so much from one diabetic person to another, and from the diabetic to the normal person, and yet show no effect as evidenced by the much narrower ergothioneine range in normal persons.

SUMMARY

A correction for the plasma effect in the diazo test for blood ergothioneine is described.

A range and mean for blood ergothioneine were established for normal subjects.

Blood from ninety-four normal persons and 107 diabetic patients was compared for mean ergothioneine content. A highly significant increase was demonstrated in the mean value for diabetic subjects.

No relationship was established between the blood ergothioneine level and any one of the following factors: diabetic state of control, duration of diabetes, age, sex, blood cholesterol, uric acid, and nonprotein nitrogen.

The author wishes to thank Dr. George Hunter for suggesting a study of the clinical significance of ergothioneine.

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FAT EMULSIONS FOR ORAL NUTRITION

I. The Absorption of Fat in the Rat

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FOR a number of years this laboratory has been interested in fat emulsions suitable for intravenous use as a means to supply abundant calories in parenteral nutrition. In addition to a need for calories in parenteral nutrition, there are many occasions on which emulsions of fat that could be given orally would be helpful. Finely emulsified fat might be advantageous over fatty foods because of greater patient tolerance and ease of administration and in the case of diseases affecting the production of pancreatic lipase it may be absorbed more efficiently. As a part of our studies on fat emulsions for oral use in man, the absorption of fat in the rat has been studied with and without emulsifiers and emulsification, and with and without various digestive enzymes.

In recent years, the accumulated data of Frazer[1] has stimulated reassessment of the mechanism of fat absorption as proposed in the lipolytic theory of Verzár and McDougall.[2] Frazer's partition hypothesis summarized states that in the presence of fatty acid, bile salt, and monoglyceride, a triglyceride (or liquid paraffin) can be readily emulsified within the intestinal lumen to particles less than 0.5 μ in diameter and then absorbed directly into the epithelial cells of the intestine, appearing finally as chylomicrons in the systemic blood. Baker[3] reported that the superficial layer of the free border of the epithelial cells of the intestine in Amphibia, reptiles, birds, and mammals contains "canals" with a maximum diameter of 0.5 μ through which these cells communicate with the intestinal lumen. Other histologic work[4] showed the apparent direct passage of fat globules into these cells.

The intestinal absorption of liquid paraffin has been denied by various workers.[5-9] Lundbaek and Maalϕe[9] used the liquid paraffin emulsions recommended by Frazer[10] and although the particles of oil ranged from 0.3 to 0.5 μ in diameter, no absorption by the rat resulted despite the maintenance, for the most part, of the particle size of the emulsion throughout the course of their experimental procedure. Several investigations,[11, 12] however, have demonstrated that small quantities of liquid paraffin are absorbed through the intestinal mucosa of rats, guinea pigs, rabbits, pigs, and human beings and may be recovered from the mesenteric lymph nodes, liver, and spleen. Since liquid paraffin is not subject to lipolysis, another mechanism of absorption must be sought for, such as direct absorption of emulsified particles of minute size.

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Crude soybean phosphatide, an effective emulsifying agent, has been stated to increase fat absorption in the rat and human being when used in high concentration in respect to the fat.[13, 14] Tidwell's work,[8] using the chylomicron count as an index of fat absorption, suggests that the choline in a phosphatide may be the active principle rather than any increase in the emulsification potential within the intestinal lumen. Tween 80 (polyoxyethylene sorbitan monooleate), another effective emulsifying agent, has also been shown to increase fat absorption in the human subject.[15]

The present study was undertaken to evaluate the absorption of fat from fat emulsions given orally, to study the particle size and stability of fat emulsions in relation to intestinal absorption, and to observe the role played by the addition of emulsifiers to fat meals. The rat was used as the experimental animal in all of the studies reported in this paper. Information obtained in a study of this type would be of assistance in directing further investigation of the use of fat emulsions for oral feeding and of the role played by the administration of exogenous emulsifiers in the digestion and absorption of fats by the human being.

EXPERIMENTAL

I. Oral Feeding Experiments.—Albino male rats of the Charles River strain (originally Wistar strain) weighing 150 to 250 grams were used. They were maintained on a stock diet of Kruncho n,* but were fasted for forty-eight hours before all procedures. All oil preparations were administered from a calibrated syringe via a stomach tube after light ether anesthesia of thirty to sixty seconds' duration. The delivery of the various preparations from the syringes used varied only ± 6 mg. maximum.

At designated intervals after intubation, the rats were sacrificed with ether. The intestinal tracts of the rats were removed after doubly ligating the esophagus in the neck, the pyloric constriction, the ileocecal junction, and the ileocolonic junction. The various segments of the tract were then cut between ligatures, emptied of their contents (which necessitated manual stripping and intralumenal irrigation for the intestine), and laid open. All mucosal surfaces were flushed carefully with an alcohol-ether mixture. When fluid was noted in the esophagus, the oropharynx was washed in retrograde fashion through the remaining stump of the esophagus.

The visceral contents obtained in this fashion were saponified with 50 per cent KOH until the nonaqueous solvent had evaporated and then were acidified with 50 per cent H_2SO_4. The saponifiable and nonsaponifiable lipids were extracted by four gentle extractions with cold, redistilled petroleum ether, and the extracts were collected into tared flasks. Filtration, followed by careful washing of the filter paper, was used when necessary to remove suspended solids and/or water from the extract. The solvents were evaporated on a steam bath and the flasks were dried to constant weight. The total saponifiable and nonsaponifiable lipids were obtained by weight. Table I summarizes the control analyses, demonstrating a recovery of at least 96 per cent ± 5 per cent (mean ± standard deviation of the mean) of the added oil preparation.

An emulsion of 40 per cent corn oil and 3 per cent of the BF2 fraction of soybean phosphatide[16] in water was prepared by homogenization in a Cherry-Burrell Junior viscolizer. The average particle size was approximately 0.5 μ in diameter. One cubic centimeter of this emulsion was intubated to rats, and the total lipid recovered two and four hours after feeding was determined. The difference between this weight of recovered lipid and the

* Gaines Division, General Foods, Kankakee, Ill.

TABLE I. RECOVERY OF OIL PREPARATIONS IN RATS SACRIFICED IMMEDIATELY AFTER ORAL FEEDING

PREPARATION	LIPID ADMINISTERED (GM.)	NUMBER OF RATS	MEAN GM. LIPID RECOVERED			MEAN TOTAL GM. RECOVERED	MEAN % RECOVERED ± S. D. OF M.	STANDARD ERROR OF MEAN (%)
			STOMACH	INTESTINE	CECUM			
None	None	6	.005	.018	.006	.029	—	—
Emulsion of corn oil using phosphatide stabilizer	.350	6	.339	.036	.006	.381	100 ± 2	1
Corn oil	.334	5	.330	.018	.008	.356	98 ± 5	2
Emulsion of corn oil using phosphatide; Gelusil also given	.350	6	.299	.058	.008	.365	96 ±5	2

A correction factor of 0.029 Gm., the mean number of grams of lipid recovered from fasted control rats, has been used in calculating the per cent recovery.

weight of lipid administered, after correcting for the amount of petroleum ether-soluble substance found in the gut of the fasted control group, was assumed to be the amount of lipid absorbed by the animals.

In similar fashion, groups of rats were fed 2.8 c.c. of a 15 per cent corn oil emulsion in 5 per cent refined gelatin* containing 0.9 per cent NaCl with a particle size of 0.5 μ, and another group was given 0.45 c.c. of corn oil followed by 0.55 c.c. of water. Since it was noted that both the 40 per cent and 15 per cent emulsions broke down almost completely within one hour in the rat stomach, 1.0 c.c. of Gelusil† was administered immediately prior to the intubation of the 40 per cent corn oil emulsion. This was found to preserve the emulsion in satisfactory fashion for at least one hour, with the majority of the particles still seen to be of the original size. However, the Gelusil interfered with the recovery of the residual lipid from the intestinal contents because of persistent "bumping" during the saponification. Therefore, the procedure was modified in these experiments by concentrating the mixtures containing Gelusil as long as possible, and then filtering and washing, using six washings of boiling alcohol. The filtrate and washes were then handled in the usual manner.

The amounts of oil contained in the various preparations administered in this study were all approximately the same, but were less than usually used in this type of study in an attempt to use approximately physiologic quantities.

II. Intestinal Instillation Experiments.—To determine more directly the possibility of absorption of oil particles by the intestine, experiments were performed in which the effects of gastric HCl, the exocrine pancreatic secretion, and bile were eliminated. Starved rats of the Charles River strain were anesthetized with ether and the proximal jejunum was ligated below all pancreatic tissue and at the ileocecal junction. The various preparations tested were instilled into the intestinal lumen through a small incision below the first ligature via a calibrated syringe and an 8 cm. long segment of ureteral catheter. Another ligature was tied immediately below the incision and the abdomen was closed. This entire procedure took approximately ten minutes, and the rats recovered from the anesthesia within fifteen minutes of the induction of anesthesia. The intestinal lumen was not irrigated, since excessive manipulation was to be avoided. The rats were sacrificed with ether four hours after the instillation, and the contents of the ligated intestine were analyzed for lipids in the described manner.

*Plazmoid, The Upjohn Company, Kalamazoo, Mich.

†Aluminum hydroxide gel and magnesium trisilicate, William R. Warner & Company, New York, N. Y.

Stability tests of emulsions stabilized with the BF2 fraction of soybean phosphatide, Span 80 (sorbitan monooleate) and Tween 80 (polyoxyethylene sorbitan monooleate), and gelatin demonstrated that only the 15 per cent oil in 5 per cent gelatin solutions were stable in such an intestinal loop for at least an hour. The average particle size of the emulsions containing gelatin was almost exactly the same after one hour as before instillation, averaging 0.5 μ in diameter.

The preparations instilled were as follows: (a) 2.0 c.c. of an emulsion of 15 per cent corn oil in 5 per cent gelatin with 0.9 per cent NaCl; (b) 0.31 c.c. of corn oil with 1.7 c.c. of 5 per cent gelatin solution; (c) 0.31 c.c. of corn oil; (d) 2.0 c.c. of an emulsion of 15 per cent liquid paraffin (heavy white mineral oil, U.S.P.) in 5 per cent gelatin; (e) 0.31 c.c. of liquid paraffin with 1.7 c.c. of 5 per cent gelatin; (f) 2.0 c.c. of an emulsion of 15 per cent peanut oil in 5 per cent gelatin with 0.9 per cent NaCl; (g) 0.31 c.c. peanut oil with 1.7 c.c. of 5 per cent gelatin with 0.9 per cent NaCl (the peanut oil emulsion had an average particle size of 1 to 3 μ in diameter and was used to elucidate any effect of this larger particle size upon intestinal absorption). Preparations a and b were also instilled into Hisaw strain rats. Table II demonstrates the range of values obtained in a typical group in this case, using the corn oil emulsion.

TABLE II. RECOVERY OF SAPONIFIABLE AND NONSAPONIFIABLE LIPID FOUR HOURS AFTER INSTILLATION OF 2.0 C.C. OF AN EMULSION OF 15 PER CENT CORN OIL IN GELATIN CONTAINING 0.9 PER CENT NaCl IN INTESTINAL LOOP EXPERIMENTS

RAT	WEIGHT (GM.)	LIPID ADMINISTERED (GM.)	LIPID RECOVERED (GM.)	LIPID ABSORBED (GM.)	LIPID ABSORBED (%)
1	160	.257	.144	.131	48
2	186	.257	.150	.125	45
3	180	.257	.174	.101	37
4	194	.257	.191	.084	30
5	212	.257	.108	.167	60
6	180	.257	.150	.125	45
7	235	.257	.170	.105	38
8	209	257	.135	.140	51
Av.	194	257	.153	.122	44

A correction factor of 0.018 Gm., the mean number of grams of total lipid recovered from the intestines of fasted control rats, has been used in calculating the lipid absorbed.

RESULTS

I. Oral Feeding.—Table III summarizes the data obtained after the oral feeding experiments. It was found that although the Gelusil preserved the physical state of the emulsion to a great extent for at least one hour, it mechanically impeded the progress of the oil into the intestine. Microscopic examination of the stomach contents of several of these animals after two hours demonstrated large globules of oil caught mechanically in the semisolid matrix of the Gelusil. The high values for the lipid recovered from the stomach confirm this observation.

The absorption of corn oil in all of the other groups, both two and four hours after feeding, was essentially the same. There is no significant difference by the "t" test (observed difference $/\sqrt{\text{standard error of the mean}\frac{2}{1} + \text{standard error of the mean}\frac{2}{2}}$, with a quotient over 2.5 to be of significance) between the absorption of oil emulsified with the BF2 fraction of soybean phosphatide or refined gelatin as compared with the corn oil alone. Since these

TABLE III. RECOVERY OF OIL PREPARATIONS IN RATS SACRIFICED TWO AND FOUR HOURS AFTER ORAL FEEDING

PREPARATION	LIPID ADMINISTERED (GM.)	NUMBER OF RATS	MEAN GM. LIPID RECOVERED			MEAN TOTAL GM. RECOVERED	MEAN GM. ABSORBED	MEAN % ABSORBED ± S. D. OF M.	STANDARD ERROR OF MEAN (%)
			STOMACH	INTESTINE	CECUM				
TWO-HOUR EXPERIMENTS									
Emulsion of corn oil using phosphatide stabilizer	.350	6	.127	.101	.007	.235	.144	38 ± 9	4
Emulsion of corn oil using phosphatide; gelusil also given	.350	7	.213	.047	.006	.266	.113	29 ± 7	3
Emulsion of corn oil using gelatin stabilizer	.360	6	.168	.054	.006	.228	.161	41 ± 9	4
Corn oil	.361	8	.159	.083	.008	.250	.140	36 ± 10	4
FOUR-HOUR EXPERIMENTS									
Emulsion of corn oil using phosphatide stabilizer	.350	7	.020	.052	.007	.079	.300	79 ± 8	3
Emulsion of corn oil using phosphatide; Gelusil also given	.350	6	.123	.032	.006	.161	.218	57 ± 13	5
Emulsion of corn oil using gelatin stabilizer	.360	6	.063	.042	.005	.110	.278	72 ± 11	4
Corn oil	.350	6	.043	.057	.012	.112	.267	70 ± 10	4

A correction factor of 0.029 Gm., the mean number of grams of total lipid recovered from fasted control rats, has been used in calculating the lipid absorbed.

emulsions broke almost completely after an hour in the stomach, this finding should be interpreted as the absence of any difference between the intestinal absorption of corn oil and corn oil with added phosphatide or gelatin solution. These emulsifiers were present in amounts, relative to the oil, sufficient to give excellent emulsions by mechanical means.

II. Intestinal Instillation.—The results of the intestinal instillation experiments are tabulated in Table IV. A very significant increase in absorption was observed with every emulsified preparation over the absorption of the unemulsified constituents used in the same proportions. In the Charles River rats, the corn oil emulsion was 26 per cent better absorbed than the corn oil with added gelatin solution; the liquid paraffin emulsion was 23 per cent better than the liquid paraffin with added gelatin; and the peanut oil, with a larger average particle size than the two preceding emulsions, was 14 per cent better absorbed than the peanut oil with added gelatin. In the Hisaw rats, the corn oil emulsion was 18 per cent better absorbed than the corn oil with added gelatin solution. By the "t" test, the quotient for the corn oil emulsion over its control is 7.2, for the liquid paraffin emulsion over its control, 5.6, for the peanut oil over its control, 4.4, and for the corn oil emulsion over its control in Hisaw rats, 6.4, all highly significant. There is no significant difference

TABLE IV. RECOVERY OF OIL PREPARATIONS IN RATS SACRIFICED FOUR HOURS AFTER INTESTINAL INSTILLATION

PREPARATION	LIPID ADMINISTERED (GM.)	NUMBER OF RATS	MEAN GM. OF LIPID RECOVERED	MEAN GM. ABSORBED	MEAN % ABSORBED ± S. D. OF M.	STANDARD ERROR OF MEAN (%)
Emulsion of corn oil using gelatin in saline	.257	8	.153	.122	44 ± 9	3
Corn oil with gelatin	.257	8	.224	.051	18 ± 9	3
Corn oil	.257	8	.233	.042	16 ± 5	2
Emulsion of liquid paraffin using gelatin	.256	6	.204	.070	25 ± 9	4
Liquid paraffin with gelatin	.265	6	.275	.008	2 ± 2	1
Emulsion of peanut oil using gelatin in saline	.240	6	.175	.083	32 ± 2	1
Peanut oil with gelatin in saline	.248	6	.218	.048	18 ± 7	3
Emulsion of corn oil using gelatin in saline; Hisaw rats used	.257	6	.172	.103	37 ± 4	2
Corn oil with gelatin in saline; Hisaw rats used	.257	6	.222	.053	19 ± 6	2

A correction factor of 0.018 Gm., the mean number of grams of total lipid recovered from the intestines of fasted control rats, has been used in calculating the lipid absorbed.

in the absorption of the corn oil emulsion between the two strains of rats. Therefore, the lipolytic digestion of the oils used, as demonstrated by the disappearance of 16 to 18 per cent of the vegetable oils and essentially none of the liquid paraffin, does not account for the disappearance of a greatly increased quantity of these same oils in a stable, emulsified form.

DISCUSSION

In these studies, purified soybean phosphatide (containing 20 per cent lecithin[17]) and gelatin did not increase the rate of absorption of oil after oral feeding. The two emulsifying agents were used in a concentration such that a good emulsion may be obtained in vitro with the amount of oil fed. This fact contrasts with the reports[8, 13, 14] of an increase of fat absorption from the intestine obtained when using amounts of phosphatide greatly in excess of that needed for stabilizing emulsions.

In the artificial conditions of the ligated intestine with the elimination of gastric, biliary, and pancreatic secretions, the definite absorption of stable, emulsified particles averaging 0.5 μ in diameter did occur. In the Charles River rats, using both the corn oil and liquid paraffin emulsions, which were similar in stability and particle size, an absorption of 23 to 26 per cent more oil was observed as compared with the unemulsified system. That this phenomenon is related to the average particle size of the emulsion is shown by the results obtained using the peanut oil emulsion in which the average particle size ranged from 1 to 3 μ, and with which only a 14 per cent increase in absorption was noted. The significant increase in the absorption of the emulsified corn oil over the unemulsified system in Hisaw rats as well demonstrates that these observations are not limited to one inbred strain.

The absorption of the emulsified liquid paraffin seems to demonstrate most aptly that this is a purely mechanical phenomenon, with the particles passing through the intestinal epithelium in some manner independent of lipolytic digestive absorption, since essentially no absorption of the unemulsified liquid paraffin was seen.

Although this work would tend to confirm a portion of Frazer's theory insofar as intestinal absorption of fat by apparently mechanical means was found, if these very artificial conditions which necessitate an emulsion of great stability with a particle size of approximately 0.5 μ were the usual occurrence in normal lipid digestion as Frazer suggests, one should occasionally see large deposits of liquid paraffin in the tissues at post-mortem examination because of the very great number of people who habitually ingest large quantities of liquid paraffin over extended periods of time. For by Frazer's partition hypothesis, the ingestion of edible oil simultaneously with liquid paraffin would give an adequate system for the absorption of an appreciable percentage of the paraffin since the required components of fatty acid, monoglycerides, and bile salts should "easily" emulsify the liquid paraffin to the required size. In this laboratory, mineral oil emulsions of suitable particle size are prepared with great difficulty when compared with vegetable oils under similar conditions.

However, there seems little doubt that very small amounts of ingested unemulsified liquid paraffin are absorbed,[11, 12] the mechanism of which is not explained by the lipolytic theory but can be explained by mechanical means if the conditions of particle size and stability of fine emulsions are favorably met.

SUMMARY

Emulsions of corn oil in water using purified soybean phosphatide or refined gelatin solution as emulsifiers were unstable in the rat stomach, breaking down within a one-hour period.

The rate and quantity of absorption of corn oil in approximately physiologic amounts by the rat after oral feeding was unaffected by the presence of refined soybean phosphatide or refined gelatin solution in amounts, relative to the quantity of oil used, sufficient to give excellent emulsions by mechanical means.

The absorption of emulsions of corn oil, peanut oil, and liquid paraffin in a refined gelatin solution after instillation into the rat intestine ligated below the pancreas and at the ileocecal junction was significantly higher than that obtained with the same unemulsified systems. This increased absorption was related to the particle size of the emulsions and their stability in the intestinal segment.

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LABORATORY METHODS

MICROPHOTELOMETRIC DETERMINATION OF GLOBULIN AND TOTAL PROTEIN IN CEREBROSPINAL FLUID OR DILUTED BLOOD SERUM

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NEWLY devised quantitative procedures for determination of globulin and total protein in cerebrospinal fluid or diluted blood serum are here described, in which methanol is used to precipitate globulin and salicylsulfonic acid to precipitate total protein, the separated proteins being held in colloidal suspension by the presence of ghatti gum and estimated directly by photoelectric (dispersimetric) measurement.

Separation of serum albumin and globulin by methanol was first effected by Liu and Wu,[1] using 30 per cent concentration at 0° C. for two or three hours. Pillemer and Hutchinson[2] separated serum albumin and globulin by means of 42.5 per cent (v/v) methanol at 0° C. and a reaction time of thirty minutes. Globulin values obtained by this method agreed closely with those given by electrophoretic analysis.

Lange[3] devised a method for the separation of globulin from cerebrospinal fluid by means of 33 per cent methanol at 37° C. overnight. The precipitated globulin was removed by centrifuging and determined by difference. Reviewing previous methods of determining globulin in cerebrospinal fluid, Lange[3] noted that all have depended upon precipitation by 50 per cent saturation with ammonium sulfate. He demonstrated that cerebrospinal fluids of low protein content yield disproportionately small amounts of precipitated globulin when ammonium sulfate is the reagent. Separation of globulin by methanol precipitation thus appears to be a superior method, although disadvantages in Lange's technique are the long period of incubation, the need for micro-Kjeldahl analysis, and the smallness of nitrogen difference which provides an estimate of the globulin.

EXPERIMENTAL

The precipitation of globulin by methanol and of total protein by salicylsulfonic acid was studied under condtions whereby the separated proteins could be estimated directly by photelometric measurement. The experimental development of these procedures is outlined, and details are given for the new techniques.

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Photoelectric Determination of Protein in Colloidal Suspensions by Light-dispersion Measurements.—The problem of turbidity measurement by optical means has been discussed by Richardson[4] who indicated the advantages of working with dilute suspensions. The methods now described involve dispersimetric determinations of protein from 0.1 mg. to 1.4 mg. in a final volume of 6.0 milliliters. Certain commercially manufactured photelometers provide such measurements, but special advantages were gained by using an optical system designed as shown in Fig. 1 and operated in a single-cell photoelectric chain similar to that described by Morris.[5]

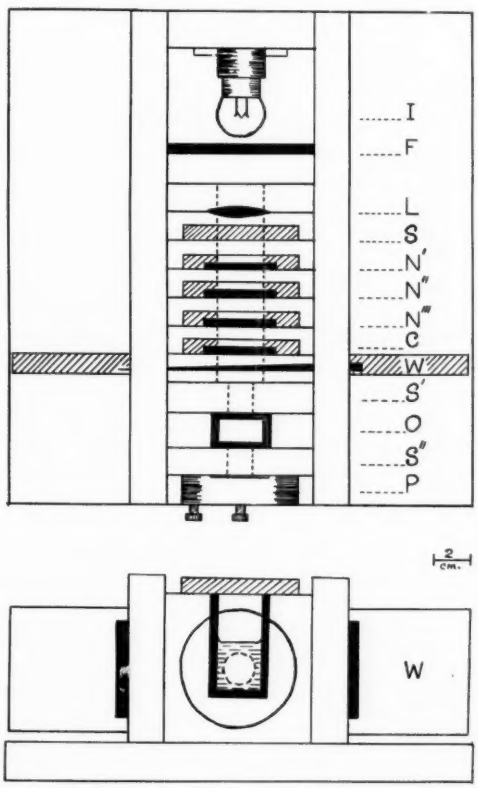

Fig. 1.—Sectional plan view (above) and end elevation (below) of the photoelectric apparatus.

I, illuminating lamp, 6 volt 6 watt, current supplied by constant voltage transformers; *F*, heat absorbing glass, 3 mm. thick; *L*, condensing lens, 5 cm. focal length; *S*, solid wood shutter, removable vertically; *N'*, *N''*, *N'''*, neutral gray screens, framed, interchangeable with others in a series of increasing density; *C*, spectral color filter, framed, interchangeable with others in a monochromatic spectral series. *W*, neutral gray optical wedge, density 0.0 - 0.2, framed, capable of lateral movement to vary the density interposed; *S'*, *S''*, fixed optical stops, 15 mm. thick, circular aperture 13 mm. diameter; *O*, plane parallel optical cell, 10 mm. optical depth; *P*, selenium barrier layer photocell, sensitive area 3 cm. diameter. The photocell is connected directly to a spot galvanometer, sensitivity 700 mm. per microampere.

Special features of the apparatus (constructed in wood) were the provision of a non-variable aperture and of a recess holding the optical cell at a fixed distance from the photocell. The optical cell was of 10 mm. optical depth, 28 mm. outside width, and 55 mm. height. The parallel focussed light beam was stopped down to 13 mm. diameter by means of a 15 mm. thick fixed stop immediately adjacent to the optical cell. A similar thick light stop was placed immediately against the other face of the optical cell and just in front of the barrier layer photocell. When a turbid fluid was contained in the optical cell, almost all of

the dispersed light was blocked by this second thick stop, and only the residual straight-through beam was allowed to illuminate the photocell. Incident light intensity was controlled by the interposition of one or more neutral gray screens whose densities were 0.1, 0.2, 0.3, etc., (as well as a spectral color filter) before the beam entered the optical cell. Refined control was effected by means of a neutral gray wedge whose optical density varied from 0.0 to 0.2, the wedge being mounted so that it could be moved by hand laterally across the

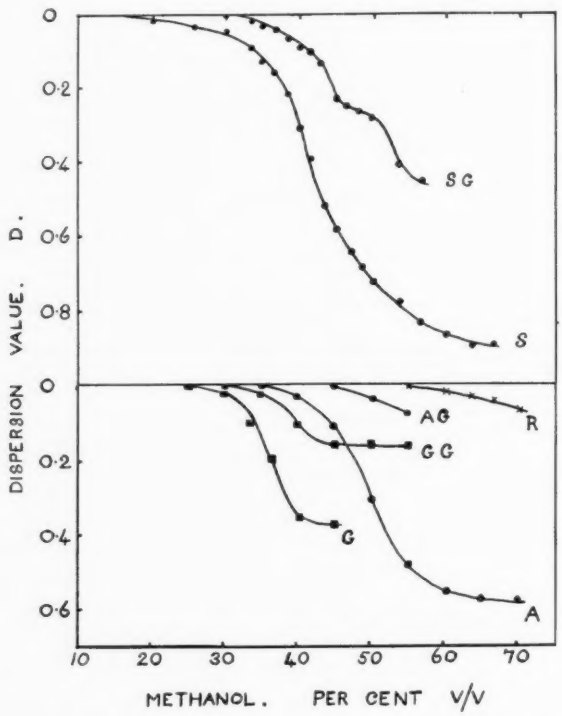

Fig. 2.—Curves correlating methanol concentrations with amounts of protein thereby precipitated, in one hour at 37° C. at pH 6.6, the separated protein being estimated by light-dispersion measurements. *S G*, diluted whole serum in the presence of ghatti gum; *S*, diluted whole serum without ghatti gum; *A G*, pure albumin in presence of gum; *A*, pure albumin without gum; *G G*, pure globulin in presence of gum; *G*, pure globulin without gum; *R*, reagent control without protein showing precipitation of ghatti gum itself.

light beam. Monochromatic violet and red wave-bands were provided by spectral color filters, having peak transmissions at $\lambda = 430$ and 700 millimicrons respectively. Optical dispersions (D) for the colloidal suspensions were determined by a technique exactly like that for extinction values (E) in translucent absorptiometry and calculated by $D_{\lambda}^{1\,cm} = \log I_o - \log I_t$. The apparatus described was equally suitable for absorptiometric or dispersimetric measurements.

Development of the Method for Determination of Globulin.—Attemps to precipitate globulin selectively from diluted sera, using methanol at differing concentrations, in one hour at 37° C., failed to demonstrate a sharply defined separation from albumin. When the mixtures were buffered with phosphate to pH 6.6 and ghatti gum was included, globulin was precipitated selectively, the precipitate was protected from flocculation, and the stable colloidal suspensions so produced were suitable for estimation by light-dispersion technique.

Fig. 2 shows typically the correlations obtained between concentrations of methanol and the amounts of protein precipitated from diluted sera, together with results of control

experiments. It was thus established that 45 per cent (v/v) methanol was effective in precipitating globulin selectively when the reaction conditions were one hour at 37° C., with phosphate buffer to pH 6.6 and 0.1 per cent ghatti gum also included in the mixture.

Other experiments demonstrated that the precipitation of globulin became complete within one hour, whether the temperature was 37° C., 20° C., or 0° C., but that the colloidal suspension developed at 37° C. possessed the greatest optical dispersive power. The mode of preparing the mixture also was investigated and a technique was devised which yielded consistently reproducible results.

Experiments in which sera were employed in varying dilution revealed that, at low dilutions, precipitation of albumin as well as globulin occurred. At higher dilutions, where the albumin concentration was not greater than 125 mg. per 100 ml., selective precipitation of globulin was successful. Confirmation that adequate diminution of the albumin concentration would allow correct globulin determinations by the methanol-dispersimetric procedure was further adduced by analyses of mixtures of pure albumin solution and whole serum, so arranged as to give varying albumin/globulin ratios. Each mixture, when diluted sufficiently to bring the albumin concentration below the critical level of 125 mg. per 100 ml., yielded correct analytical values for globulin.

Development of the Method for Determination of Total Protein.—The use of salicylsulfonic acid was adopted by King and Haslewood[6] for their determination of cerebrospinal fluid protein. Mawson[7] improved the technique and applied it also to serum, though he did not make use of ghatti gum which had been employed previously by Looney and Walsh[8, 9] as a protective colloid. Cipriani and Brophy[10] described a microphotelometric technique but they also failed to use a dispersing agent.

A method for determination of total protein, analogous to that for globulin, was devised using 5 per cent salicylsulfonic acid reagent which formed uniform colloidal suspensions provided sufficient (0.2 per cent) ghatti gum was present. Using the same buffered ghatti gum reagent as devised for the globulin technique, the pH of the final mixture became 1.03. No difference could be demonstrated between reactions carried out at 20° C. and at 37° C. and precipitation was complete within two minutes. Using the technique described, correct values were obtained for total protein concentrations when solutions containing albumin and globulin in widely varying proportions were analyzed.

METHODS FOR THE DETERMINATION OF TOTAL PROTEIN AND GLOBULIN

Reagents.—1. Salicylsulfonic acid, analytical grade, 5 per cent (w/v) solution in water.

2. Methanol, analytical grade.

3. Sodium chloride, 0.9 per cent (w/v) solution in water.

4. Ghatti gum, purified powder. Twenty grams of commercial gum are ground in a mortar, then stirred into 100 ml. cold water in a beaker and allowed to stand overnight. A further 100 ml. water are then added and the beaker is heated in a boiling water bath. The viscous solution is thoroughly stirred until dissolution of the gum is fairly complete, after which it is strained and squeezed through cotton gauze to remove coarse impurities, then reheated and filtered overnight without suction through eight layers of cotton gauze fitted into a large Buchner funnel. The gum solution is then poured slowly with constant stirring into 9 volumes absolute ethanol. The mixture is filtered with suction through a fine-grade paper in a Buchner funnel; the precipitated gum is washed with a little 90 per cent (v/v) ethanol, then absolute ethanol, and finally is dried in vacuo over calcium chloride in a desiccator and stored.

5. Buffered ghatti gum reagent. Purified gum powder, 1.5 grams, is dissolved by stirring with 150 ml. hot water. The cooled solution is transferred to a 250 ml. graduated flask; 15 ml. 0.067M disodium hydrogen phosphate and 25 ml. 0.067M potassium dihydrogen phosphate are added and the mixture is brought to 250 ml. with water. The pH of the reagent is checked as 6.6 using bromthymol blue indicator; the reagent is then stored with a few drops of chloroform as preservative.

Procedure for Total Protein.—Blood serum is diluted 1 in 60 with sodium chloride solution to bring the protein concentration within measurable range; cerebrospinal fluid need be diluted only if the protein concentration exceeds 140 mg. per 100 milliliters. Volumes of 3.0 ml. salicylsulfonic acid solution and 2.0 ml. buffered ghatti gum reagent are transferred to a test tube, 5 in. by ½ in., and mixed by closing the tube and inverting it six times. One milliliter of protein solution is then added, the tube again closed, and the contents mixed immediately by inverting ten times. After five minutes the fluid is transferred to a 10 mm. optical cell and its light-dispersion value determined in the photoelectric apparatus, using spectral red light and a protein-free blank solution in the reference cell. The protein concentration is then obtained from a standard reference graph prepared appropriately and correlating red light-disperision values ($D^{1\,cm}_{700}$)with known protein concentrations. If the protein concentration is low, a dispersimetric value ($D^{1\,cm}_{430}$) of greater magnitude may be obtained advantageously by using spectral violet light and the result read from the appropriate graph.

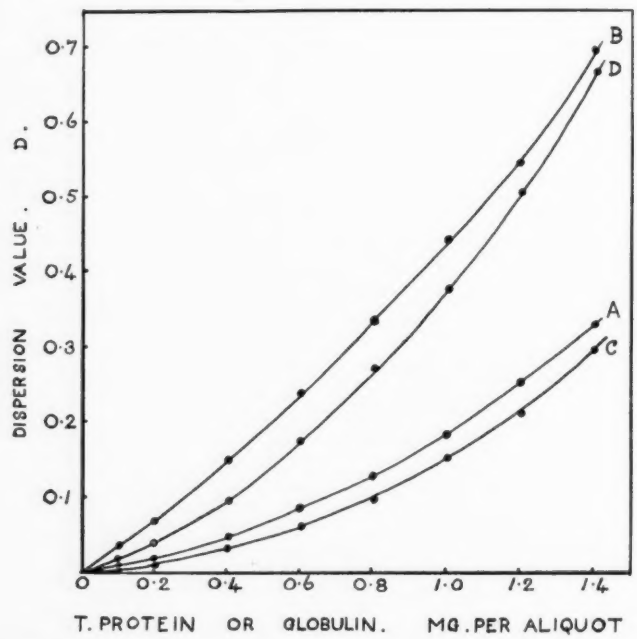

Fig. 3.—Standard reference graphs correlating light-dispersion values with protein concentrations. *A*, total protein, spectral red; *B*, total protein, spectral violet; *C*, globulin, spectral red; *D*, globulin, spectral violet.

When red light is used and the material contains hemoglobin or bilirubin, no correction is necessary for light absorption; but if violet light is selected, the gross value for $D^{1\,cm}_{430}$ is corrected by simple deduction of the extinction value ($E^{1\,cm}_{430}$) for a 1 in 6 dilution of the material in sodium chloride solution, before the protein concentration is read from the standard reference graph.

Procedure for Globulin.—It is necessary to ensure that the concentration of albumin in the solution which is being analyzed for globulin is not greater than 125 mg. per 100 milliliters. A dilution of blood serum in 0.9 per cent sodium chloride solution, 1 in 60, is usually suitable. For cerebrospinal fluid it is convenient to prepare a dilution so that the

total protein (already determined) does not exceed 125 mg. per 100 milliliters. The globulin concentration must also be within measurable range, i.e. not greater than 70 mg. per 100 milliliters.

Volumes of 1.0 ml. ghatti gum reagent, 0.3 ml. sodium chloride solution, and 2.7 ml. methanol are transferred to a test tube, 5 in. by ½ in.; the tube is closed and inverted six times to mix the contents. Two milliliters of protein solution are then added and the tube immediately is inverted ten times to ensure complete mixing. The tube is then stoppered to prevent evaporation and placed in a water bath at 37° C. for one hour. The fluid is then transferred to an optical cell and its light dispersion value determined in the photoelectric apparatus using spectral red light and a protein-free blank solution in the reference cell. The result is obtained from the appropriate standard reference graph unless the globulin concentration is low. In such a case, the light-dispersion value is redetermined using spectral violet light and the result is obtained from the corresponding graph. (Fig. 3.) If the material contains violet light-absorbing pigment, the extinction value for a 2 in 6 dilution in sodium chloride solution is deducted from the observed gross dispersion value before referring to the graph for the corresponding globulin concentration.

Preparation of Standard Reference Graphs.—Serum of known total protein content (micro-Kjeldahl) is used to prepare a series of dilutions in sodium chloride solution extending over the range 10 to 140 mg. protein per 100 milliliters. As the graph is a curve, eight points are plotted to ensure accurate correlation between light-dispersion readings and total protein concentrations. Dispersions are determined for both red and violet wave-bands.

For globulin determinations, serum (low in albumin) of known globulin and albumin content is diluted to provide a series of eight solutions whose globulin concentrations extend over the range 5 to 70 mg. globulin per 100 ml. and whose albumin concentrations nowhere exceed 125 mg. per 100 milliliters. Curves are drawn to correlate globulin concentrations with dispersimetric values for both red and violet wave-bands. It is recommended that the globulin content of the serum used for standardization be determined by the method of Cohn and Wolfson[11] whose procedure for separating globulins makes use of 26.88 per cent sodium sulfite at room temperature.

The precise form of the standard curves depends upon the mean particle size in the suspensions—also to some extent upon the design of the photoelectric apparatus employed. Curves should, therefore, be established appropriate to each instrument in use as well as to the particular analytical techniques.

In the suspensions, the particle size is controlled by the ghatti gum, both in the globulin and in the total protein techniques, so that the standard graphs are reliable as long as the same reagents are used. When the stock ghatti gum powder is renewed, the standard reference graphs should be re-established.

SUMMARY

Consideration is given to the problem of estimating globulin and total protein in low concentrations such as are found in cerebrospinal fluid.

A method is presented for the precipitation of globulin by 45 per cent (v/v) methanol at pH 6.6 in the presence of ghatti gum in one hour at 37° C. Albumin is not precipitated under these conditions provided its concentration is below the critical level of 125 mg. per 100 milliliters.

A similar procedure is described for the precipitation of total protein by salicylsulfonic acid in the presence of ghatti gum in five minutes at room temperature.

Details are given of a photoelectric apparatus whereby the precipitated proteins, held in colloidal suspension by the gum, are determined directly by light-dispersion measurements. Dispersimetric values are correlated with glob-

ulin and with total protein concentrations by means of empirically established curves. Spectral red light is ordinarily used but when greater sensitivity is required the wave-band is advantageously changed to spectral violet.

The analytical procedures presented are applicable to diluted blood serum as well as to cerebrospinal fluid and provide reliable results for quantities of protein from 0.1 mg. to 1.4 mg. per aliquot analyzed.

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A SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ACETALDEHYDE IN BLOOD

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DURING a study of the mechanism of action of tetraethylthiuramdisulfide in this laboratory it became necessary to determine blood acetaldehyde levels. The Stotz[1] technique was reviewed and was found to give satisfactory results. However, this method had the drawback of being time consuming. Since no other reliable and rapid technique was available, a procedure was developed which facilitated the simultaneous handling of a number of acetaldehyde determinations. Separation of acetaldehyde from blood was accomplished by diffusion in a Conway cell. A semicarbazide solution was used as the reactant and the resulting acetaldehyde semicarbazone was determined spectrophotometrically with a Beckman DU spectrophotometer. When a 2 ml. blood specimen is analyzed, acetaldehyde in concentrations as low as 0.2 μg per milliliter can be determined with an accuracy of plus or minus 3.5 per cent.

EXPERIMENTAL

Development of a Spectrophotometric Method for the Determination of Acetaldehyde.—Semicarbazide is a well-known agent for the isolation and identification of aldehydes and ketones. In the customary procedure, the resulting semicarbazone is crystallized and the melting point determined. Evans and Gillam[2] studied the absorption spectra of a number of semicarbazones. These authors reported that semicarbazones gave an appreciable absorption in the ultraviolet region at very low concentrations. Conant[3] studied the kinetics of aldehyde-semicarbazide reactions and reported that pH 7 was the optimum for the formation of acetaldehyde semicarbazone. These findings were the basis for the development of the present method.

The Absorption Spectrum of Acetaldehyde: To 3 ml. of a 0.0067 molar semicarbazide hydrochloride solution in a pH 7 phosphate buffer,† 1 ml. of a solution containing 30 μg of acetaldehyde was added (water cooled to below 10° C. in preparation of acetaldehyde solution). This was allowed to warm to room temperature and was diluted to volume in a 10 ml. volumetric flask. The absorption spectrum of the resulting semicarbazone was determined with a Beckman DU spectrophotometer between wave lengths 220 and 350 mμ, as shown in Fig. 1. It will be noted that the maximum absorption occurred at a wave length of 224 millimicrons. At this wave length, the minimal workable slit width was found to be 0.86 millimeter. Hence, this wave length and slit width were used in the determinations.

Determination of a Standard Curve: Into nine 10 ml. volumetric flasks were pipetted 3 ml. of the buffered semicarbazide solution. Into three of these were added 1, 2, or 3 ml. of a 15.6 μg per milliliter cooled solution of acetaldehyde. All nine flasks were allowed

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†Buffer contains 0.06 molar NaH_2PO_4 and 0.14 molar Na_2HPO_4.

to warm to room temperature and were diluted to volume and the optical densities were determined on the Beckman DU spectrophotometer, using as a blank 3 ml. of the buffered semicarbazide solution diluted to 10 milliliters. At the wave length and slit width given, a linear relationship was shown to exist between the optical density and concentration, as indicated in Fig. 2.

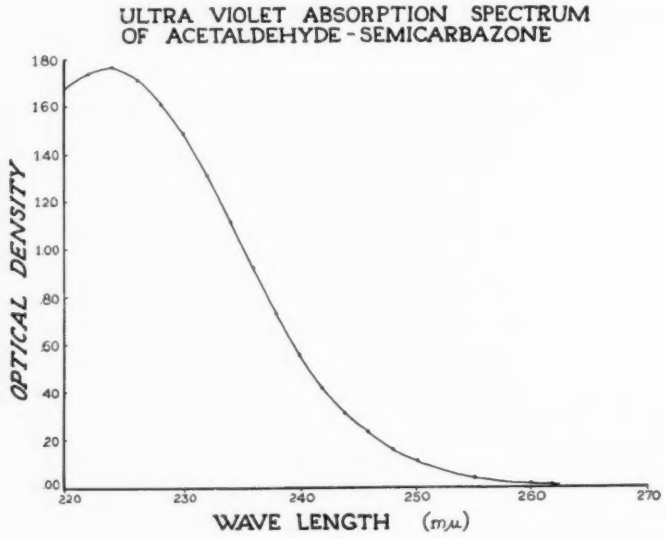

Fig. 1.

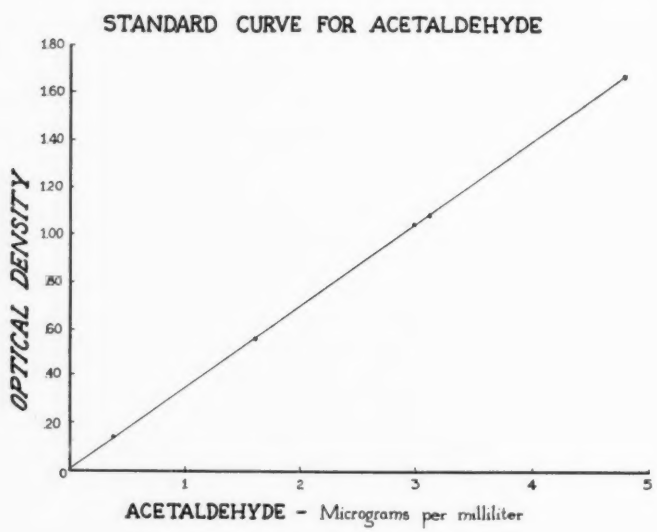

Fig. 2.

The Stability of Acetaldehyde-Semicarbazone: An acetaldehyde-semicarbazone decay curve was determined in the following manner. Three milliliters of the buffered semicarbazide reactant solution were pipetted into the center chamber of each of three Conway cells. Into the outer chamber of each cell was pipetted 1 ml. of a standard acetal-

dehyde solution containing 8.52 μg per milliliter. The cells were placed in an oven at 28° C. for ninety minutes. The contents of the center chambers were then quantitatively transferred to a 10 ml. volumetric flask and diluted to volume. The optical densities at 224 mμ were then determined immediately and at hourly intervals for the following six hours, as shown in Table I. It was observed that while there is a loss of less than 0.5 per cent in the first hour, by the second hour a 5 per cent loss has occurred. Maximum loss had occurred by the fourth hour. Repetition of this experiment with smaller concentrations of acetaldehyde gave similar results. For optimal results, the spectrophotometric determinations should be carried out within ninety minutes of removal of cells from the oven.

TABLE I. THE STABILITY OF ACETALDEHYDE SEMICARBAZONE

TIME	RECOVERY (μG*)	DEVIATION FROM ORIGINAL VALUE (%)
Initial	8.52	—
1 hr.	8.50	0.23
2 hr.	8.07	5.2
3 hr.	7.85	7.7
4 hr.	7.75	9.0
5 hr.	7.77	8.8
6 hr.	7.75	9.0

*Average of three determinations.

Separation of Acetaldehyde From Blood.—In the Stotz technique the acetaldehyde is separated from blood by distillation. This procedure requires the attention of the analyst over a period of time when a number of samples are to be determined. We have adapted the technique described by Conway in the determination of ammonia[4] for the separation of acetaldehyde from blood or plasma. A cell of 7.5 cm. outside diameter with an inner concentric chamber 3 cm. in diameter proved to be most satisfactory for the 2 ml. samples generally used. The diffusion rate of acetaldehyde from blood was determined on a number of specimens and ninety minutes was found to be the optimum time for samples containing up to 15.6 μg per milliliter.

Description of the Method.—The following technique provided the most rapid and accurate procedure for the determination of acetaldehyde. Three millimeters of the buffered semicarbazide HCl solution are pipetted into the center chamber. The top of the outer wall of the cell is greased with Lubriseal. Two milliliters each of 2/3N sulfuric acid and 10 per cent sodium tungstate are added to the outer chamber just prior to addition of the blood sample. Two milliliters of well-shaken fresh blood are added to the outer chamber and the cover is immediately put in place. The blood and protein precipitants are mixed by gently rotating the cell. The cells are then placed in an oven with the temperature controlled between 28° and 30° C. and the diffusion is allowed to proceed for ninety minutes. At the end of this time the contents of the center chamber are quantitatively transferred to 10 ml. volumetric flasks and brought to volume with distilled water. The optical densities of these solutions are read on a Beckman DU spectrophotometer at wave length 224 mμ and at slit width 0.86 millimeter. The reference blank for these readings is 3 ml. of the semicarbazide solution diluted to volume in a 10 ml. volumetric flask. From the optical densities obtained, the corresponding micrograms of acetaldehyde can be read from the standard curve. The final value is then multiplied by five in order to correct for dilution.

Recovery of Acetaldehyde From Water and Blood.—Preparation of standard solutions: 1 ml. of reagent grade acetaldehyde kept at less than 12° C. was pipetted into a 1 liter volumetric flask (partially filled with cold water) and diluted to volume with distilled water at the same temperature. This solution which contained 780 μg milliliter was further diluted, 1:50, 1:100, and 1:250 to give solutions of 15.6, 7.8, and 3.1 μg per milliliter. Blood samples were prepared by addition of varying quantities of the standard aqueous

solutions to freshly drawn normal human blood. Normal blood acetaldehyde levels by our method range from 40 to 110 μg per cent. Table II illustrates recoveries of acetaldehyde from blood and water at three concentrations in each medium.

TABLE II

(A) THE RECOVERY OF ACETALDEHYDE FROM WATER

ACETALDEHYDE ADDED (μG)	RECOVERY (μG*)	PER CENT ERROR
3.10	3.06	- 1.3
7.80	7.93	+ 1.6
15.60	15.40	- 1.3

(B) THE RECOVERY OF ACETALDEHYDE FROM BLOOD

ACETALDEHYDE ADDED (μG)	BLOOD BLANK	RECOVERY (μG†)	DIFFERENCE	PER CENT ERROR
1.85	1.07	2.90	1.83	- 1.1
7.80	1.07	8.77	7.70	- 1.3
15.60	1.07	15.51	15.44	- 1.0

*Average of three determinations.
†Average of six determinations.

DISCUSSION

A method for the rapid determination of blood acetaldehyde levels has been described. In this laboratory one analyst has completed as many as forty-eight determinations in a five-hour period.

This method allows considerable variation in sample and reactant volumes. We recommend that with this size cell at least 2 ml. of semicarbazide solution be employed in order to increase the surface area of the reactant solution and thus decrease the time for diffusion. The smaller cell described by Conway[4] can be used with smaller volumes. We have found blood samples from 0.5 to 2.0 ml. to be adequate for the amount of acetaldehyde encountered in normal blood and the blood of patients undergoing Antabuse therapy. The range of normal acetaldehyde levels in human blood was the same whether we used laked blood, a protein-free filtrate of blood, or blood from which the proteins were precipitated in the outer chamber of the cell. Normal acetaldehyde blood levels as determined by our method ranged from 40 to 110 μg per cent in fourteen healthy persons in the fasting and nonfasting states. Stotz[1] reported the normal free acetaldehyde content of blood to be from 22 to 37 μg per cent in seven fasting individuals.

The formation of semicarbazones is a nonspecific reaction shared by a number of aldehydes, ketones, and sugars. Therefore, there are theoretically a number of substances in the blood which could interfere with the determination of acetaldehyde by this method. Diffusion eliminates interference from all non-volatile agents. Of the volatile substances possibly present in human blood, acetone would be the most likely to interfere. As yet no one has shown the presence of measurable quantities of acetone in the blood and it can only arise in significant amounts through the decomposition of acetoacetic acid (this occurs in the lung and bladder). This decomposition has been minimized by keeping the diffusion temperature at 29° C., which is subbody temperature and well below the boiling point of acetone (56.5° C.). Moreover, the rate of reac-

tion of acetaldehyde with semicarbazide is 500 times that of acetone.[3] As further indication of the noninterference of ketone bodies, acetaldehyde was determined on the blood of five patients with a positive test for acetone in the urine (four of them strongly positive). All five determinations fell in the normal range of 40 to 110 μg per cent.

At the present time, over 400 blood acetaldehyde levels have been determined in this laboratory and the method has been found to be satisfactory.

SUMMARY

A new method for the determination of blood acetaldehyde levels has been described.

The method is accurate to about plus or minus 3.5 per cent.

The method is especially convenient when a large number of determinations are to be made simultaneously.

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PRESERVATION OF ALBUMIN-FORTIFIED AND UNFORTIFIED BLOCKING-TYPE Rh ANTISERUM WITH SODIUM AZIDE

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THE first Rh antibody to be described,[1] and the type contained in the Rh typing antisera commonly used in laboratory tests until 1944-45, was of the agglutinin or saline-acting type, active against saline suspensions of red blood cells. It was observed early that such antisera were relatively unstable during storage and deteriorated rapidly when contaminated with many of the bacteria commonly present in the laboratory. Furthermore, the presence of bacterial contaminants frequently resulted in development of nonspecific agglutinating properties of the antisera.

As laboratory tests for determination of the Rh factor became more commonly employed, the need for satisfactory, stable typing sera became increasingly important, and various chemical agents have been studied in attempts to find a satisfactory bactericidal or bacteriostatic preservative. Apparently some preservative agents exert a deleterious effect on the Rh agglutinin. Thus, Waller[2] found that Merthiolate inhibited or destroyed the activity of the Rh agglutinin and Cameron and Diamond[3] reported that an anti-Rh serum containing 0.1 per cent "Compound I" (carboxylmethoxylamine hemihydrochloride) showed loss of both titer and avidity after thirty days of storage at room temperature.

The successful use of sodium azide (NaN_3) in preserving liquid complement[4] suggested the possibility of the use of this agent in the preservation of Rh typing sera. In previously reported experiments conducted at the Army Medical Department Research and Graduate School,[5, 6] it was found that sodium azide, in 0.1 per cent concentration, was a satisfactory bacteriostatic agent for preservation of a typing serum containing the agglutinin type of Rh antibody. The chemical exerted no deleterious action on this particular type of antibody even when preserved serum was stored at various temperatures for a period of twenty-five weeks.

Following discovery of the blocking Rh antibody,[7] and demonstration of its in vitro agglutinative activity when diluted in plasma or serum albumin,[8] sera containing this type of antibody received preference as Rh typing reagents because of their greater stability and availability. With the development of these superior Rh typing sera, the need for a preservative appeared less acute. However, it frequently has been observed that the activity of even albumin-fortified, blocking-type antisera was reduced or completely destroyed by bacterial contaminants. Aside from the report by Diamond and Abelson[9] that

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Merthiolate "while inactivating anti-Rh agglutinins, appears not to affect" Rh blocking antibodies, little attention apparently has been paid to the effect of preservatives on Rh antibodies of this type. This presentation is a report of a study of the preservative effect of sodium azide on a blocking-type Rh antiserum with and without added bovine albumin.

MATERIALS AND METHODS

A human serum with a high content of D (Rh_o) blocking antibody was sterilized by filtration, diluted with sterile normal human serum to a final agglutinative titer of approximately 1:512, and divided into two portions. Bovine albumin, Fraction V (Armour), was added to one portion to a final concentration of 25 per cent (w/v). The albumin-fortified and the unfortified portions each were divided into three equal parts and in each case sodium azide was added to 0 per cent, 0.05 per cent, and 0.1 per cent concentration respectively. Each of these portions then was subdivided into two parts; one half was maintained as a sterile specimen and the other half was seeded with a mixed suspension of known bacteria to a concentration of approximately 1,000,000 organisms per milliliter of serum. The bacteria employed were common laboratory contaminants obtained from samples of 30 per cent bovine albumin and of human serum which had been exposed to the air. The organisms were identified as gram-positive bacilli, streptococci, and staphylococci.

Each serum specimen was titered for Rh agglutinative activity, and contamination or sterility was determined by cultural methods. Each sample then was divided into three aliquots which were stored at refrigerator, room, and incubator (37° C.) temperatures respectively.

The titrations were performed by adding 0.1 ml. volumes of a 2 per cent suspension of pooled D (Rh_o) positive red blood cells to 0.2 ml. quantities of twofold serial dilutions of the test sera. Twenty per cent bovine albumin was employed as the diluent in preparing both the cell suspensions and the serum dilutions. These serum-cell suspensions then were incubated at 37° C. for forty-five minutes, centrifuged, and macroscopically observed for agglutination. In accordance with commonly accepted standards,[10] an antiserum titer of 1:32 was selected as the minimum adequate for routine diagnostic use.

RESULTS

Rh antibody titrations were run on duplicate samples of the antiserum preparations at frequent intervals during a period of seventy-two weeks. The rate of deterioration (decrease in titer) in most instances was slow, indicating the relative stability of the blocking-type Rh antibody. Results of the titrations on the unfortified and albumin-fortified specimens performed following twelve, twenty, twenty-five, thirty-seven, and seventy-two weeks of storage are presented in Tables I and II.

Effect of Storage Temperature.—The rate of decrease in agglutinative activity (titer) of the unfortified serum specimens was markedly dependent upon the storage temperature (Table I). Refrigerated specimens retained adequate titer (1:32 or higher) throughout the period of study, seventy-two weeks. With the exception of the non-sodium azide treated, contaminated samples, serum specimens stored at room temperature retained adequate titers for thirty-seven weeks. In samples stored at 37° C., a sharp drop in activity was apparent at twenty to twenty-five weeks.

The agglutinative activity of the albumin-fortified specimens was much less affected by storage temperature (Table II). While evidence of a definite

TABLE I. TITERS* OF BLOCKING-TYPE ANTI-D (Rh_0) SERUM SPECIMENS AS AFFECTED BY BACTERIAL CONTAMINATION, NaN_3 CONCENTRATION, TEMPERATURE, AND PERIOD OF STORAGE (SERUM SPECIMENS NOT FORTIFIED WITH BOVINE ALBUMIN)

STORAGE TEMPERATURE	NAN_3 CONCENTRATION (%)	STERILE SPECIMENS — PERIOD OF STORAGE (WEEKS)						CONTAMINATED SPECIMENS† — PERIOD OF STORAGE (WEEKS)					
		0	12	20	25	37	72	0	12	20	25	37	72
37° C.	0.10	512	64	16	16	4	Neg.	*512*	*64*	*64*	*8*	Neg.	Neg.
		512	64	16	16	2	Neg.	*512*	*128*	*64*	*16*	*32*	*Neg.*
	0.05	512	64	16	8	2	Neg.	*512*	*64*	*64*	*16*	*8*	*Neg.*
		512	64	32	4	2	Neg.	*512*	*128*	*64*	*16*	*4*	*Neg.*
	0.00	512	128	16	16	2	Neg.	*512*	*32*	*16*	*4*	Neg.	*Neg.*
		512	128	16	8	2	Neg.	*512*	*64*	*16*	*1*	Neg.	Neg.
Room (20-25° C.)	0.10	512	256	64	64	16	4	*512*	256	128	128	128	8
		512	256	64	64	32	4	*512*	256	128	128	128	16
	0.05	512	256	128	64	32	2	*512*	128	128	128	64	4
		512	256	64	64	32	4	*512*	*256*	*256*	32	*Hem.*	*Hem.*
	0.00	512	256	64	128	32	2	*512*	*256*	*Hem.*‡	*Hem.*	*Hem.*	*Neg.*
		512	128	128	128	32	2	*512*	*256*	*Hem.*	*Hem.*	*Hem.*	Neg.
Refrigerator (2-10° C.)	0.10	512	256	256	256	128	64	*512*	256	128	128	128	64
		512	256	256	256	128	64	*512*	256	128	256	128	64
	0.05	512	256	256	128	128	32	*512*	256	256	128	128	64
		512	256	256	128	64	32	*512*	256	128	256	128	64
	0.00	512	512	128	128	64	32	*512*	256	128	128	128	64
		512	512	256	256	64	32	*512*	256	128	128	128	64

*Reciprocals of greatest dilutions giving macroscopic agglutination of test cells.

†Entries in italics denote viable contaminants were recovered by culture at time of titration.

‡Serum specimen hemolyzed test cells at time of titration.

decrease in titer was obtained in each instance, all specimens except those stored for seventy-two weeks at 37° C. retained adequate titer throughout the study.

Effect of Sodium Azide.—In no instance was evidence obtained of any deleterious effect of sodium azide on the activity of the antiserum employed. At all storage temperatures, titers of sodium azide treated, sterile samples were essentially comparable to those of the unpreserved samples. In one instance (unfortified serum stored at room temperature), the results indicated that a sodium azide concentration of 0.05 per cent was inadequate to maintain effective bacteriostasis.

Effect of Contaminants.—The apparent lack of effect of the bacterial contaminants on any of the refrigerated specimens is somewhat misleading since viable organisms were recovered from only one sample following more than twelve weeks of storage.

A deleterious effect of the contaminants was clearly evident in serum specimens stored at room temperature. Contaminated samples not treated with sodium azide and not fortified with albumin exhibited marked hemolytic activity following twenty weeks of storage at room temperature. Hemolysin production by the contaminants was only partially controlled by sodium azide in 0.05 per cent concentration.

The contaminants exerted a slight but distinct effect on the activity of unpreserved, unfortified specimens stored at 37° C. The effect was only that of a decrease in the titer of these specimens; apparently the storage temperature

TABLE II. TITERS* OF BLOCKING-TYPE ANTI-D (Rh_0) SERUM SPECIMENS AS AFFECTED BY BACTERIAL CONTAMINATION, NaN_3 CONCENTRATION, TEMPERATURE, AND PERIOD OF STORAGE (SERUM SPECIMENS FORTIFIED WITH BOVINE ALBUMIN, 25 PER CENT W/V)

STORAGE TEMPERATURE	NaN_3 CONCENTRATION %	STERILE SPECIMENS PERIOD OF STORAGE (WEEKS)						CONTAMINATED SPECIMENS† PERIOD OF STORAGE (WEEKS)					
		0	12	20	25	37	72	0	12	20	25	37	72
37° C.	0.10	512	64	128	64	32	16	*512*	128	128	64	32	16
		512	256	128	64	64	16	*512*	128	128	64	32	16
	0.05	512	256	128	64	64	16	*512*	256	256	128	32	16
		512	256	128	64	32	32	*512*	256	128	64	32	16
	0.00	512	256	128	64	32	16	*512*	*512*	*128*	*128*	*32*	*32*
		512	256	128	64	32	16	*512*	*256*	*128*	*64*	*64*	*16*
Room (20-25° C.)	0.10	512	256	128	128	64	32	*512*	128	256	128	32	N.D.‡
		512	256	128	128	64	32	*512*	256	128	128	64	
	0.05	512	256	128	128	64	64	*512*	256	128	64	32	N.D.
		512	128	128	128	64	32	*512*	256	128	128	32	
	0.00	512	128	128	128	64	32	*512*	*256*	*128*	*128*	64	N.D.
		512	256	128	128	64	32	*512*	256	128	128	64	
Refrigerator (2-10° C.)	0.10	512	256	128	128	64	64	*512*	64	128	128	64	N.D.
		512	256	128	256	64	64	*512*	64	128	128	64	
	0.05	512	256	128	128	128	64	*512*	*256*	128	256	64	N.D.
		512	256	128	128	128	32	*512*	256	128	256	64	
	0.00	512	512	128	128	128	64	*512*	256	128	256	64	N.D.
		512	256	128	64	128	64	*512*	128	128	128	64	

*Reciprocals of greatest dilutions giving macroscopic agglutination of test cells.

†Entries in italics denote viable contaminants were recovered by culture at time of titration.

‡Titrations not done because of depletion of sample.

was not favorable for the production of hemolysin. At this temperature, either sodium azide or albumin fortification appeared adequate to prevent the harmful effect of the contaminants.

Effect of Bovine Albumin Fortification.—The activity of the albumin-fortified specimens was less affected by any of the experimental conditions (contamination, time, and temperature of storage) than was that of the unfortified specimens. In each instance the fortified specimens retained adequate titer for a period of thirty-seven weeks and, although the data were not complete, available evidence indicates that such specimens retain adequate titer for a period of seventy-two weeks when stored at either room or refrigerator temperatures.

Bovine albumin in the concentration employed undoubtedly exerts appreciable bacteriostatic activity. This was evidenced by frequent failure to recover viable organisms from the fortified specimens and by the absence of the development of hemolytic activity in any of the fortified samples. It is not considered justifiable to assume, however, that a chemical preservative is unnecessary in albumin-fortified Rh typing sera. The concentration of albumin used in this study (25 per cent w/v) is considerably greater than generally is employed in preparation of Rh typing reagents, and the bacteriostatic effect of a lower concentration undoubtedly would be less.

SUMMARY

A study is reported of the preservative effects of sodium azide on unfortified and bovine albumin-fortified blocking-type D (Rh_0) antiserum stored at re-

frigerator, room, and 37° C. temperatures over a period of seventy-two weeks.

Sodium azide, in a concentration of 0.1 per cent, was found to exert no deleterious effect on the blocking-type antibody and to be a satisfactory bacteriostatic agent in both unfortified and albumin-fortified serum specimens.

The agglutinative activity of albumin-fortified serum specimens was much less affected by bacterial contaminants, temperature, and time of storage than was that of unfortified samples.

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A PRESSURE PUMP FOR ADMINISTERING INTRA-ARTERIAL OR INTRAVENOUS FLUIDS

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CLEVELAND, OHIO

IN CATHETERIZATION of the left side of the heart in man and in animals it is necessary to maintain a constant pressure of fluid within the catheter above that present in the artery. To obviate the necessity of using a positive pressure bulb we designed a simple pressure pump which maintains a constant air pressure which may be regulated over a range from 0 to 400 mm. Hg. The pump is a reciprocating one of 250 c.c. per minute capacity and driven by a

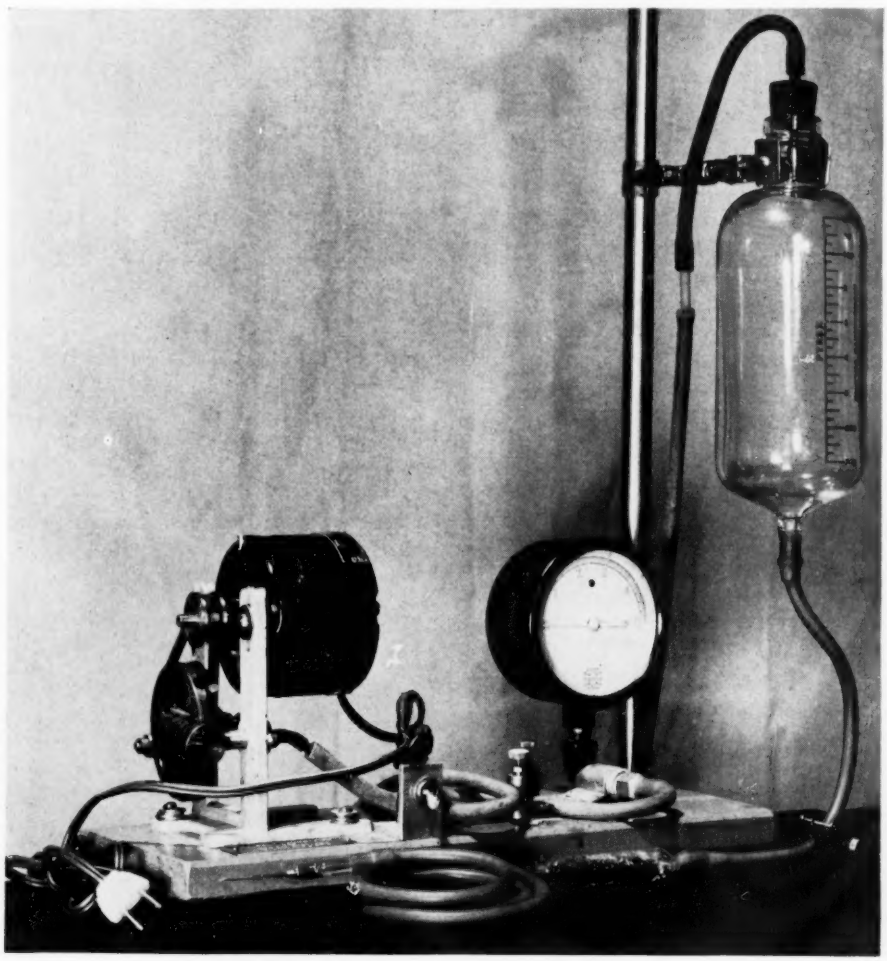

Fig. 1.—A photograph of apparatus assembled.

From the Department of Medicine, Western Reserve University, the Medical Service, Cleveland City Hospital, and the Rand Institute for Medical Research.
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1/150 horse power motor. A pressure gauge of 5 lb. per square inch range is used to measure the pressure (1 lb. per square inch = 50 mm. Hg). By use of a needle valve the desired pressure may be maintained constant and uniform. Instead of a Murphy drip, a safety float valve is used to automatically seal the tubing when the injection fluid is exhausted and thus prevent air entering the artery or vein.

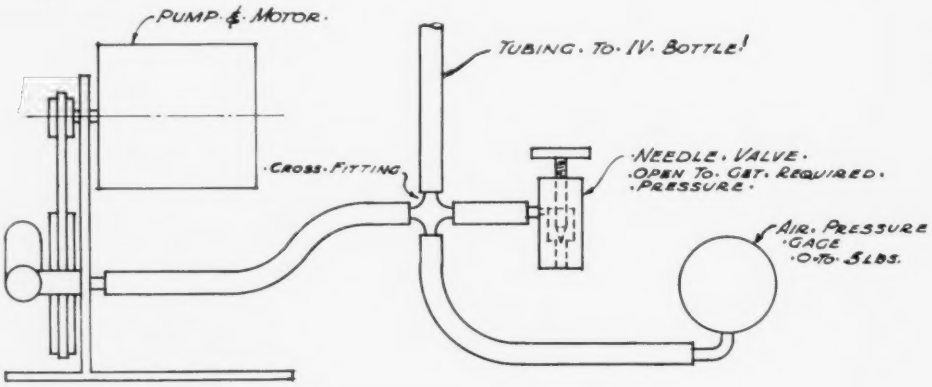

Fig. 2.—A detailed drawing of the pressure pump.

Fig. 1 is a photograph of the apparatus and Fig. 2 is a detailed drawing of the pump assembly. This pressure pump is easily constructed from readily available materials, and both in the laboratory and in the operating room it has proved to be a valuable aid in maintaining a constant pressure during the injection of either intravenous or intra-arterial fluids.

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